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HAWAII AGRICULTURAL EXPERIMENT STATION,
J. M. WESTGATE, Agronomist in Charge.

Bulletin No. 37.

AMMONIFICATION AND NITRIFICATION IN HAWAIIAN SOILS.

BY

W. P. KELLEY,
Chemist.

UNDER THE SUPERVISION OF
OFFICE OF EXPERIMENT STATIONS,
U. S. DEPARTMENT OF AGRICULTURE.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
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HAWAII AGRICULTURAL EXPERIMENT STATION, HONOLULU.

[Under the supervision of A. C. TRUE, Director of the Office of Experiment Stations, United States Department of Agriculture.]

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[1] Resigned October 27, 1914.

LETTER OF TRANSMITTAL.

HONOLULU, HAWAII, *January 10, 1914.*

SIR: I have the honor to submit herewith and recommend for publication as Bulletin No. 37 of the Hawaii Agricultural Experiment Station, a paper on Ammonification and Nitrification in Hawaiian Soils, prepared by Dr. W. P. Kelley, chemist of the station. The nitrogen compounds which occur in soils and the modifications which they undergo are of great importance in practical agriculture. In many Hawaiian soils the conditions which influence the form and changes of these compounds are somewhat unusual. A study of the factors which modify ammonification and nitrification is therefore of great scientific and practical importance. It is believed that a distinct contribution to the knowledge of these processes and also to an understanding of the significance of the lime-magnesia ratio, particularly as it is related to changes in the nitrogen compounds of the soil, is made in this bulletin.

Respectfully,

E. V. WILCOX,
Special Agent in Charge.

Dr. A. C. TRUE,
Director Office of Experiment Stations,
U. S. Department of Agriculture, Washington, D. C.

Publication recommended.
A. C. TRUE, *Director.*

Publication authorized.
D. F. HOUSTON, *Secretary of Agriculture.*

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AMMONIFICATION AND NITRIFICATION IN HAWAIIAN SOILS.

INTRODUCTION.

The importance of bacteria in soils has become generally recognized. In contrast to the extreme chemical view formerly held it is now believed that the biological activities going on in soils are of more fundamental importance, and that as a result of bacterial action the minerals become more soluble and chemical transformations are brought about in the organic and inorganic constituents.

Soils, therefore, are no longer looked upon as dead reservoirs of plant food, but, on the contrary, as teeming with organized life. Various chemical substances, the degree of porosity, the moisture content, and other factors, all exert important influence on the activity of soil organisms. For these reasons the application of fertilizers, tillage, crop rotation, etc., directly affect the soil organisms, and therefore, indirectly, the chemical changes. But the real seat of bacterial action is the organic matter, and it is this part of the soil that undergoes the greatest change as a result of their action.

Practically all organic substances occurring in soils undergo decomposition to some degree with the consequent formation of a great variety of chemical compounds, principally organic in nature. Some of these products exert marked chemical action on the mineral constituents; certain of them are toxic both to the higher plants and to the bacteria themselves, while others serve as nutrients to the higher plants. In the end, however, these become converted into carbon dioxid, water, ammonia, nitrate, free nitrogen gas, etc.

Phases of soil bacteriology which have received a great amount of attention are ammonification and nitrification. Soil nitrogen exists principally in complex, insoluble, protein-like combinations, in which form it is unavailable to the higher plants, but by the action of bacteria ammonia is split off, which then is oxidized to nitrate.

Formerly, much emphasis was placed on the numbers of organisms present in soils. More recently, however, it has been found that the physiological efficiency of the organisms in different soils varies so greatly that now it is more common to measure the products of their action on nitrogenous substances rather than to base conclusions on the number of organisms present.

In considering the various factors that influence the growth of crops in Hawaii, it is inevitable that attention should be turned to certain phases of the nitrogen question. In the first place, Hawaiian soils generally contain relatively large amounts of nitrogen, on the average at least twice as much as mainland soils. Nevertheless, enormous amounts of nitrogenous fertilizers are applied to the land now in cultivation, and in some instances it has been found very profitable to use nitrogenous fertilizers on soils which contain large amounts of nitrogen. The low availability of the nitrogen in the soils emphasizes the need of a better understanding of the bacterial processes going on.

In the second place, many Hawaiian soils contain very high percentages of clay and little gravel or sand, and, therefore, are very close textured; consequently aeration is poor. Such lands, especially when plowed for the first time, are exceedingly inert. In the pineapple section of Oahu it is necessary to allow the new lands to lie fallow, with occasional cultivation, for a period of several months after the first plowing before planting, but it has been found that the growth of crops is normal and satisfactory on the new lands immediately after plowing, where brush and other refuse had been burned. Heat, therefore, seems to accomplish the same effect as continued aeration.

In the third place, Hawaiian soils are extremely abnormal in mineral composition. Various substances, such as ferric and aluminum hydrate, the oxids of manganese, titanium compounds, etc., are present in large amounts. Besides, carbonates, except in a few localities, are present in extremely small amounts. It is commonly held that the presence of calcium carbonate is essential to successful crop production, for the reason that nitrification is believed to be dependent on it for the maintenance of neutral conditions. How far other bases can take the place of calcium carbonate is not fully known.[1] The relative and absolute amounts of lime and magnesia in Hawaiian soils vary greatly, but generally magnesium occurs in considerably larger amounts than calcium. The lime-magnesia ratio is a question of much interest among soil investigators at the present time, but the bearings of this ratio on bacterial action have not been thoroughly studied. In view of the large amounts of lime, some of which is highly magnesian in character, now being applied to soils, a study of ammonification and nitrification as affected by variations in this ratio is of general interest.

In the investigation reported in this bulletin the effects of certain factors on nitrification and ammonification have been studied, but many organic forms of nitrogen are also known to be available to the higher plants, and other factors frequently complicate the subject so

[1] See Ashby, Jour. Agr. Sci., 2 (1907), pp. 52–67.

as probably to render the nitrification process of less relative importance than has been frequently assumed. The nitrifying bacteria ar quite sensitive to lack of aeration, the presence of stagnant water, acidity, certain chemical substances, and the like, but there are instances in Hawaii in which the intensity of nitrification appears to bear no relation whatever either to the growth of crops or to the presence of chemical substances that are definitely poisonous to some agricultural crops. Hence the amount of nitrate and the intensity of nitrification in a soil should not be considered as forming adequate measures of the availability of nitrogen. The results obtained in nitrification and ammonification experiments, therefore, should be interpreted with caution, and every known condition and factor, as well as the crops to be grown, must be given due weight before anything like a satisfactory practical conclusion can be drawn.

AMMONIA AND NITRATE CONTENT OF UNCULTIVATED SOILS.

One of the first questions studied in this investigation has reference to the rates at which nitrification and ammonification take place in soils in situ. This is obviously important in establishing a basis for the comparison of different treatments and as offering some suggestion on the management of these soils. It is of special interest, moreover, for the indirect evidence furnished regarding the form of nitrogen that is probably utilized by the different plants growing on these soils.

While much study has hitherto been devoted to nitrification and ammonification in cultivated soils, so far as the writer has been able to find from literature at hand, very little investigation has been made on nitrification in sod lands, or soils lying long uncultivated. Certain references occur in the literature concerning the low nitrification going on in forest soils. Grandeau,[1] for instance, found no nitrate in certain forest soils, while Weis [2] reported considerable nitrate in the moor and forest soils of Denmark. Ritter [3] found little tendency toward nitrate formation in moor soils, as a rule, although he detected small amounts of nitrate in certain cases. Petit,[4] on the other hand, found pronounced evidence of nitrification in a decidedly acid forest soil deficient in lime. The nitrate content of peaty soils in America, on the other hand, is sometimes almost negligible. Jodidi [5] failed to detect nitrate in certain Michigan peats. It is generally known, moreover, that practically no nitrification takes place in the subsoils of humid climates.

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[1] Jour. Agr. Prat., n. ser., 13 (1907), pp. 645, 646.
[2] Forstl. Forsögsv., 2 (1908), No. 2, pp. 257–296.
[3] Internat. Mitt. Bodenk., 2 (1912), No. 5, pp. 411–428.
[4] Ann. Sci. Agron., 4. ser., 2 (1913), II, No. 4, pp. 397, 398.
[5] Michigan Sta. Tech. Bul. 4 (1909).

In this investigation a wide range of moisture and other conditions were met with. Samples were drawn from pasture lands, submerged soils supporting a crop of rice and taro, similar lands left to dry but not cultivated after the crops had been harvested, forest and fern jungles, abandoned pineapple and cane fields, and the like. Some of the samples were drawn at times when the soil was almost air dry, while others were taken when the moisture content was near the optimum for plant growth. The plants growing on these soils represent a considerable range of species.

When possible the analyses were made immediately after taking the samples, so as to render unnecessary the use of antiseptics. Whenever the sample could not be analyzed immediately a few cubic centimeters of chloroform was added. Nitrate was determined by leaching 100-gram portions with water and then determining the nitrate dissolved by the use of the phenol disulphonic acid method; ammonia was determined by distilling 100-gram portions in copper flasks after adding magnesium oxid.

The results of determinations of nitrate and ammonia nitrogen in uncultivated soils by these methods are given in the following table:

Nitrate and ammonia nitrogen in uncultivated soils.

[Parts per million.]

Lab. No.		Crop and locality.	Nitrate nitrogen.	Ammonia nitrogen.
229	Soil	Pasture, Wahiawa	0.2	25.2
230	Subsoil	do	.2	19.6
233	Soil	do	.1	21.0
234	Subsoil	do	.1	11.2
235	Soil	do	.4	16.8
236	Subsoil	do	.2	14.0
273	Soil	Citrus orchard, station	.4	11.2
292	do	Rice, Waikiki	.5	2.0
293	Subsoil	do	3.0	7.0
300	Soil	Pasture, Kaneohe	Trace.	19.6
301	Subsoil	do	.0	11.2
302	Soil	Abandoned pineapple field, Kaneohe	.5	19.6
303	Subsoil	do	.7	16.0
306	Soil	Pasture, Kaneohe	.9	22.4
307	Subsoil	do	.3	15.4
310	Soil	do	.2	16.0
312	do	do	.1	21.0
313	do	Pasture and guava, Kaneohe	.4	26.6
315	do	Guava, Kaneohe	.3	18.2
328	do	Pasture, Kohala	1.3	12.6
330	do	Pasture, Wahiawa	1.3	14.0
334	do	Rice, Fort Shafter	.0	11.2
335	do	Coconut grove, Kailua	2.5	[1]
336	Subsoil	do	.7	[1]
337	Soil	Rice land, Kailua	15.0	[1]
338	Subsoil	do	5.7	[1]
341	Soil	Coffee, Maunawili	.5	[1]
342	Subsoil	do	2.2	[1]
417	Soil	Pasture Waipio	.4	7.7
449	do	Panicum, Glenwood	.6	14.0
450	do	Fern forest, Glenwood	.3	11.2
451	do	Sugar cane, Glenwood	.3	16.8
452	do	Fern forest, Glenwood	.2	26.6
454	do	do	.2	42.0
456	do	Sugar cane, Glenwood	.3	5.6
457	do	Pasture, Glenwood	.4	18.2
458	do	Ferns, etc., Glenwood	Trace.	30.8
486	do	Pasture, Kunia	6.0	[1]
488	do	Pasture, Wahiawa	1.6	[1]

[1] Not determined.

The very low nitrate content in all the samples examined with the exception of Nos. 337, 338, and 486 will at once be noted. Of these, No. 337 was taken in the Kailua district of Oahu from a rice field just after harvest, and, being a recently reclaimed tule marsh, the soil contains a high percentage of organic matter and is much more porous than the average island soil. No. 486 was taken from an old pasture in the Kunia district, near the lands now devoted to pineapples by the Hawaii Preserving Co. This soil is notably silty and is also much more porous than the island soils generally. Therefore all the uncultivated soils examined which contained any considerable amount of nitrate are porous and hence permit of considerable aeration without cultivation.

Not all the porous and organic soils in the island contain such amounts of nitrate when uncultivated. Nos. 449 to 458 are exceptionally well aerated soils, but none of these contained more than traces of nitrate. Climatic factors in this instance probably determine the low nitrate content. The samples were taken from the Hilo district of Hawaii where rainy weather prevails a large portion of the year, but when these soils are brought into warmer conditions nitrification has been found to set in.

The ammonia content of these soils, as shown in the table, is abnormally high, ranging from 2 to 42 parts per million. In general the ammonia content of soils elsewhere is much less than the nitrate content,[1] which is accounted for by the fact that the ammonia is nitrified almost as fast as it is formed. In Hawaiian soils, however, particularly where cultivation is not practiced, nitrification takes place very slowly, in many instances scarcely at all, which, as will be shown subsequently, is due to the lack of aeration. Ammonification, on the other hand, not being so dependent on aeration and also being less sensitive to other adverse conditions, goes on more or less uninterruptedly, with the result that ammonia accumulates to some extent.

As pointed out above, nitrification was formerly believed to be necessary to the growth of plants. Experiments are not wanting, however, which show that other forms of nitrogen can be assimilated. In a number of instances it has been found that ammonia and organic nitrogen compounds can be utilized as advantageously as nitrate. From the experiments of Müntz,[2] Laurent,[3] Griffiths,[4] Pitsch,[5] Hutchinson and Miller [6] and others, working under sterile conditions, it has been shown that ammonium compounds can be assimilated to a

[1] Fraps found that some Texan soils also contain relatively large amounts of ammonia. Texas Sta. Bul. 106 (1908).

[2] Compt. Rend. Acad. Sci. [Paris], 109 (1889), p. 646.

[3] Ann. Inst. Pasteur, 3 (1889), p. 362.

[4] Chem. News, 64 (1891), p. 147.

[5] Landw. Vers. Stat., 34 (1887), pp. 217–258; 42 (1893), pp. 1–95.

[6] Jour. Agr. Sci., 3 (1909), pp. 179–194.

considerable extent by different plants. Moreover, Krüger[1] found that mustard, oats, and barley assimilate ammonia equally as well as nitrate, while potatoes prefer ammonia. From his experiments he concluded that nitrification is not so necessary for cultivated plants as has been supposed.

In 1910 [2] the writer showed that ammonia is greatly superior to nitrate in the nutrition of rice. It was found, for instance, that rice made very poor growth when nitrate was the only source of combined nitrogen present, but different ammonium compounds proved well suited to the plant. More recently Hutchinson and Miller[3] have shown that a considerable variety of organic nitrogen compounds can be assimilated and transformed into protein by peas. Certain organic nitrogen compounds, however, prove to be ill suited to the nutrition of peas.

Likewise, Schreiner et al.[4] have demonstrated that creatinin occurs in notable amounts in fertile soils, and is as valuable in the nutrition of wheat as nitrate.

From the data above submitted it is at once apparent that plants, growing on the uncultivated soils of Hawaii, must necessarily depend largely on forms of nitrogen other than nitrate, for not only is nitrate practically absent, but as will be shown subsequently, nitrification in many instances will not take place until aerated conditions have been maintained for a period of weeks; and the vigorous growth of practically all the uncultivated species in the islands and of such crops as rice, taro, and bananas, each of which is frequently grown under conditions which prevent nitrification, furnishes abundant evidence of the availability of the nitrogen present, and points conclusively to the dependence on forms other than nitrate.

Since ammonia nitrogen was found in these soils in considerable amounts, and ammonification can take place under the prevailing conditions, it seems justifiable to believe that ammonia is an important source of available nitrogen to the plants growing here, and that ammonification is of far greater importance than nitrification.

SOIL AERATION.

The importance of aeration in soils is generally recognized. In general, the degree of aeration depends upon the porosity and water content, and can be greatly increased by tillage. Notwithstanding the importance of oxygen in soils, and the fact that aeration stimulates bacterial action, the specific effects resulting from aeration are far from being adequately understood.

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[1] Landw. Jahrb., 34 (1905), p. 761.
[2] Hawaii Sta. Bul. 24.
[3] Jour. Agr. Sci., 4 (1912), pp. 282-302.
[4] U. S. Dept. Agr., Bur. Soils Bul. 83 (1911).

In view of the inactive state of nitrification in the uncultivated soils of Hawaii, considerable interest is attached to a study of the effects produced by aeration. This phase of the question has been taken up from two slightly different standpoints; first, with reference to the nitrate and ammonia content of soils cultivated without any special reference to the time and mode of tillage, and second, with reference to the possibility of the uncultivated soils containing agents that hinder nitrification.

The results of the determinations of nitrate and ammonia nitrogen in different cultivated soils are given in the following table:

Nitrate and ammonia nitrogen in cultivated soils.

[Parts per million.]

Lab. No.		Crop and locality.	Nitrate nitrogen.	Ammonia nitrogen.
274	Soil	Citrus orchard, station	10.0	11.2
288	do	Corn, station	4.7	16.8
289	do	do	1.8	12.6
290	do	Citrus orchard, station	14.5	15.4
291	Subsoil	do	6.5	16.0
304	Soil	Pineapple, Kaneohe	10.0	12.6
305	Subsoil	do	12.6	21.0
308	Soil	do	17.0	19.6
317	do	No crop, Kaneohe	15.4	12.6
319	do	do	9.7	18.2
326	do	Corn, Kohala	19.0	15.4
327	do	Pineapple, Kohala	10.0	12.6
329	do	No crop, Waipio	75.0	33.6
331	do	No crop, Helemano	32.0	28.0
332	do	No crop, Fort Shafter	3.0	8.4
333	Subsoil	do	1.5	8.4
339	Soil	No crop, Kailua	10.0	[1]
340	Subsoil	do	5.0	[1]
416	Soil	Pineapples, Wahiawa	40.0	11.9
428	do	Corn, Glenwood	7.2	64.0
455	do	Lilies, Glenwood	4.7	21.0
459	do	No crop, Glenwood	1.0	4.2
485	do	Pineapples, Kunia	10.0	[1]
487	do	Pineapples Wahiawa	10.8	[1]

[1] Not determined.

A comparison of the above data with that in the previous table indicates that, when aerated conditions are brought about in Hawaiian soils, nitrification generally becomes active. Ammonification was also stimulated by tillage. However, as previously stated, nitrification is at a low ebb in certain soils, although well aerated. The above table shows that soil No. 459 contained only one part of nitrate per million. This soil had been thoroughly tilled for several months and contained a large amount of organic matter. The low nitrification taking place here appears to be due to climatic factors rather than the absence of the nitrifying organisms and is being further investigated.

The composition of the mineral matters in the above soils varies enormously. No. 329 is highly manganiferous; No. 485 contains about 20 per cent titanic oxid; No. 288 contains a large excess of magnesia, while a majority of these soils are highly ferruginous, containing on the average about 20 per cent ferric oxid. Since the

nitrate content bears no definite relation to the amount of the above-named mineral constituents, and in a number of instances was equal to that found in soils elsewhere, it is safe to conclude that the abnormal mineral composition of Hawaiian soils does not prevent active nitrification and ammonification. The temperature being near the optimum for bacterial action greatly encourages nitrification when the other conditions are suitable.

AMMONIFICATION AND NITRIFICATION IN SOILS PREVIOUSLY UNCULTIVATED.

The inert character of the virgin soils of Hawaii has already been referred to. Moreover, heavy applications of various fertilizers, including nitrate, often fail to induce vigorous growth of pineapples on the new lands. In investigating this phenomenon, various treatments have been applied, including aeration for different lengths of time, the application of lime, burning, and partial sterilization. Large samples of soil were taken from uncultivated fields, and at the same time samples of corresponding soil cultivated at intervals for 10 months without having any crop growing thereon. At the time of sampling the nitrate and ammonia were as follows:

Nitrate and ammonia nitrogen in cultivated and uncultivated soils.

[Parts per million.]

Lab. No.	Condition.	Nitrate nitrogen.	Ammonia nitrogen.	Lab. No.	Condition.	Nitrate nitrogen.	Ammonia nitrogen.
329	Cultivated	75.0	33.6	417	Uncultivated	0.4	7.7
330	Uncultivated	1.3	14.0	487	Cultivated	10.8	[1]
416	Cultivated	40.0	11.9	488	Uncultivated	1.6	[1]

[1] Not determined.

The above data again show that aeration greatly stimulates both nitrification and ammonification, and that the uncultivated soils contain an extremely low nitrate content.

EFFECTS OF BRIEF AERATION.

Further investigation of the effects produced by aeration led to a study of nitrification and ammonification at various intervals after the samples were drawn. The samples were divided into different portions. One of each was spread out in the laboratory to dry. The nitrate and ammonia were determined in these portions at intervals, as shown in the table following.

Effects of aeration on the content of nitrates and ammonia nitrogen in soils.

[Parts per million.]

Lab. No.	Condition when brought to laboratory.	Days after bringing to laboratory.	Nitrate nitrogen.	Ammonia nitrogen.	Lab. No.	Condition when brought to laboratory.	Days after bringing to laboratory.	Nitrate nitrogen.	Ammonia nitrogen.
329	Cultivated	None.	75.0	33.6	417	Uncultivated	315	0.5	50.4
329	do	28	160.0	56.0	485	Cultivated	None.	10.0	(1)
330	Uncultivated	None.	1.3	14.0	485	do	232	11.0	36.4
330	do	28	85.0	30.8	486	Uncultivated	None.	6.0	(1)
416	Cultivated	None.	40.0	11.9	486	do	232	5.0	40.6
416	do	14	57.5	12.5	487	Cultivated	None.	10.8	(1)
416	do	315	62.0	44.8	487	do	200	9.0	61.6
417	Uncultivated	None.	.4	7.7	488	Uncultivated	None.	1.6	(1)
417	do	14	.6	12.2	488	do	200	1.2	58.6

[1] Not determined.

During the drying out, ammonification took place to a considerable extent but at a more vigorous rate in the cultivated than in the uncultivated soils. Nitrification also took place in the cultivated soils, but with the exception of No. 330, was inactive in the uncultivated soils. The moisture content of these soils at the time of sampling was about one-half saturation, but, of course, rapidly decreased. Nevertheless, considerable nitrification took place in soils Nos. 329, 330, and 416 during the first three weeks.

The portions to be used in studying the effects produced by aeration for a brief time were thoroughly mixed upon reaching the laboratory, placed in large fruit jars, sterile water added in sufficient amounts to bring the moisture content to two-thirds saturation, then loosely covered with cotton plugs, and kept at from 27° to 30° C. in a dark closet. At various intervals portions were withdrawn with a sterile spatula, and the nitrate and ammonia determined. The results follow:

Ammonification and nitrification in soils after short periods of aeration.

[Parts per million.]

Lab. No.	Previous condition.	Days after taking from field.	Nitrate nitrogen.	Ammonia nitrogen.	Gain(+) or loss(−).	
					Nitrate nitrogen.	Ammonia nitrogen.
329	Cultivated	None.	75.0	33.6		
329	do	14	140.0	42.0	+ 65.0	+ 8.4
329	do	32	220.0	22.4	+145.0	− 11.2
329	do	46	220.0	33.6	+145.0	.0
330	Uncultivated	None.	1.3	14.0		
330	do	14	13.0	11.2	+ 11.7	− 2.8
330	do	32	14.6	11.2	+ 13.3	− 2.8
330	do	46	7.0	28.0	+ 5.7	+ 14.0
416	Cultivated	None.	40.0	11.9		
416	do	14	70.0	19.6	+ 30.0	+ 7.7
416	do	28	92.0	14.0	+ 52.0	+ 2.1
416	do	42	90.0	28.0	+ 50.0	+ 16.1
417	Uncultivated	None.	.4	7.7		
417	do	14	.6	22.4	+ .2	+ 14.7
417	do	28	13.0	42.0	+ 12.6	+ 34.3
417	do	42	12.0	42.0	+ 11.6	+ 34.3
485	Cultivated	None.	10.0	(1)		
485	do	14	18.0	11.4	+ 8.0	
485	do	28	27.5	18.2	+ 17.5	[2] + 6.8
485	do	42	31.5	7.0	+ 21.5	[2] − 4.4
485	do	101	56.0	5.6	+ 46.0	[2] − 5.8
486	Uncultivated	None.	6.0	(1)		
486	do	14	18.0	7.5	+ 12.0	
486	do	28	25.0	12.6	+ 19.0	[2] + 5.1
486	do	42	34.5	14.0	+ 28.5	[2] + 6.5
486	do	101	70.0	16.8	+ 64.0	[2] + 9.3

[1] Not determined.

[2] Gain or loss after 14 days.

From the above table it will be seen that nitrification and ammonification were stimulated by the brief aeration and that a maximum nitrate and ammonia content was reached in about four weeks, except in the case of soils Nos. 485 and 486. It is of special interest that the intensity of both nitrification and ammonification in the uncultivated soils was considerably less in every instance except No. 486 than in the corresponding cultivated soil, which again points to the fact that tillage, for a short time only, is not sufficient to cause vigorous bacterial action. Soil 486 at first appears to be an exception, but it should be remembered that aerated conditions ensue in this soil without cultivation. The data obtained from it, therefore, the more strongly emphasizes the fact that aeration not only supplies the oxygen necessary to bacterial action, but also brings about other changes, directly or indirectly, which appear to be fundamental to vigorous bacterial action.

EFFECTS OF LIME, INFUSIONS, AND THE LIKE, ON AMMONIFICATION AND NITRIFICATION.

There is a popular belief in Hawaii that the sod lands are acid, due to anaerobic fermentation, and that the acidity can be overcome (neutralized) by bringing about aerated conditions for a sufficient length of time. Thus it is that the farmer explains the beneficial effects of tillage. On the other hand, bacteriologists hold that bacteriotoxins may accumulate in soils in certain conditions, and that the nitrifying organisms either may not be present in soils long remaining under anaerobic conditions, or lose in part their physiological activity. In order to throw some light on these questions infusions from a soil containing vigorous nitrifying and ammonifying floras were added to portions of the cultivated and uncultivated soils, and in addition, dried blood at the rate of 2 grams and calcium carbonate at the rate of 1 gram per 100 grams of soil. After bringing to optimum moisture with sterile water, the soils were kept in tumblers at temperatures from 27° to 30° C. for 7 days in the ammonification experiments, and 21 days in the nitrification experiments. At the end of these periods the ammonia and nitrate were determined, as shown in the table following.

Ammonification and nitrification in cultivated and uncultivated soils.

[Parts per million.]

Lab. No.	Previous condition.	Treatment.	Ammonia nitrogen found.	Nitrate nitrogen found.	Gain (+) or loss (−).	
					Ammonia nitrogen.	Nitrate nitrogen.
329	Cultivated	None	37.8	135.0	+ 4.2	+ 60.0
329	do	Infusion	40.6	143.0	+ 7.0	+ 68.0
329	do	2 gm. dried blood	1,519.0	131.0	+1,485.4	+ 56.0
329	do	2 gm. dried blood+1 gm. $CaCO_3$	1,503.0	129.0	+1,469.4	+ 54.0
329	do	2 gm. dried blood+1 gm. $CaCO_3$+ infusion.	1,532.0	132.0	+1,498.4	+ 57.0
330	Uncultivated	None	11.9	22.4	− 2.1	+ 21.1
330	do	Infusion	12.6	20.8	− 1.4	+ 19.5
330	do	2 gm. dried blood	925.0	9.9	+ 911.0	+ 8.6
330	do	2 gm. dried blood+1 gm. $CaCO_3$	1,219.0	9.5	+1,205 0	+ 8.2
330	do	2 gm. dried blood+1 gm. $CaCO_3$+ infusion.	1,144.0	11.0	+1,130.0	+ 9.7
416	Cultivated	None	10.5	78.0	− 1.4	+ 38.0
416	do	1 gm. $CaCO_3$	6.3	82.0	− 5.6	+ 42.0
416	do	2 gm. dried blood	1,486.0	186.0	+1,474.1	+146.0
416	do	2 gm. dried blood+1 gm. $CaCO_3$	1,472.0	178.0	+1,460.1	+138.0
417	Uncultivated	None	5.9	2.5	− 1.8	+ 2.1
417	do	1 gm. $CaCO_3$	5.6	4.2	− 2.1	+ 3.8
417	do	2 gm. dried blood	1,034.0	3.5	+1,026.3	+ 3.1
417	do	2 gm. dried blood+1 gm. $CaCO_3$	1,130.0	2.2	+1,122.3	+ 1.8

The above results show that previous cultivation produced remarkable effects on ammonification and nitrification, especially the latter. Thus it was found that the nitrates in cultivated soils Nos. 329 and 416 without treatment increased in 21 days from 75 and 40 parts to 135 and 78 parts per million, respectively, while the nitrate in the corresponding uncultivated soils Nos. 330 and 417 increased from 1.3 and 0.4 parts to only 22.4 and 2.5 parts, respectively. Expressing these results in another way, cultivated soil No. 329 gained 60 parts per million of nitrate nitrogen, while the corresponding uncultivated soil No. 330 gained only 21.1 parts, and cultivated soil No. 416 gained 38 parts per million, while the uncultivated soil No. 417 gained only 2.1 parts.

The addition of active infusions brought about only slight increase in nitrification, while the addition of dried blood caused a slight decrease in nitrates in soil No. 329, and a considerably larger decrease in soil No. 330. On the other hand, nitrification in soil No. 416 was greatly stimulated by the addition of dried blood, but no effects were noticed in the corresponding uncultivated soil No. 417. Only slight effects were produced by the addition of calcium carbonate, thus showing that acidity is not the cause of the low nitrification in these soils.

Turning to the effects produced on ammonification, we find that neither the addition of active infusions nor of lime produced any effects, but that the ammonification of dried blood was active in every case, although proceeding with more vigor in the cultivated soils.

As further showing that something more than the mere supplying of free oxygen and active infusions is necessary in order to bring about nitrification in these soils, the experiments reported in the following table were carried out with soils Nos. 487 and 488. Soil No. 487 came from a field which had been thoroughly cultivated for a period of months, but for three weeks immediately previous to sampling excessively wet weather had prevailed, during which time the soil had been saturated practically all the time. Soil No. 488 represents the corresponding uncultivated soil.

Ammonification and nitrification in soils after continuous rains.

[Parts per million.]

Lab. No.	Previous condition.	Treatment.	Nitrate nitrogen found.	Ammonia nitrogen found.	Gain (+) or loss (−).	
					Nitrate nitrogen.	Ammonia nitrogen.
487	Cultivated	None	13.2	53.0	+2.4	0.0
487	do	2 gm. dried blood	7.7	281.0	−3.1	+228.0
487	do	2 gm. dried blood+2 gm. $CaCO_3$	13.5	239.0	+2.7	+186.0
487	do	2 gm. dried blood+2 gm. $CaCO_3$+ infusion.	11.5	235.0	+0.7	+182.0
488	Uncultivated	None	2.7	60.2	+1.1	0.0
488	do	2 gm. dried blood	2.6	303.1	+1.0	+242.9
488	do	2 gm. dried blood+2 gm. $CaCO_3$	7.2	227.5	+5.6	+167.3
488	do	2 gm. dried blood+2 gm. $CaCO_3$+ infusion.	5.5	226.1	+3.9	+165.9

Here we see that ammonification took place, although not so actively as in the soils previously discussed, and that neither lime nor active infusions brought about any increase over that which occurred without them. Practically no nitrification took place in any portion of the cultivated or uncultivated soil. Thus while the previous cultivation had affected the nitrate content to a slight extent, the beneficial effects produced were very soon destroyed in the saturated condition. These soils contain a very high clay content and a small amount of humus, and the clay is exceedingly deflocculated. Continued rains, therefore, cause packing and bring about anaerobic conditions.

In the following series 10 cubic centimeters of infusion, obtained by vigorously shaking for 10 minutes 100 grams of uncultivated soil No. 417 with 200 cubic centimeters sterile water, were added to 100 grams of the cultivated soil No. 416 both with and without dried blood and calcium carbonate. At the same time infusions from the cultivated soil were added to portions of the uncultivated soil. After the usual incubation periods, ammonia and nitrate were determined, with the results shown in the table following.

Ammonification and nitrification as affected by infusion from the cultivated and uncultivated soils.

[Parts per million.]

Lab. No.	Previous condition.	Treatment.	Ammonia nitrogen found.	Nitrate nitrogen found.	Gain (+) or loss (−).	
					Ammonia nitrogen	Nitrate nitrogen.
416	Cultivated	None	10.5	78	− 1.4	+ 38.0
416	do	Infusion from No. 417	8.4	79	− 3.5	+ 39.0
416	do	2 gm. dried blood+1 gm. $CaCO_3$	1,472.0	178	+1,460.1	+138.0
416	do	2 gm. dried blood+1 gm. $CaCO_3$+infusion from No. 417.	1,437.0	157	+1,425.1	+117.0
417	Uncultivated	None	5.9	2.5	− 1.8	+ 2.1
417	do	Infusion from No. 416	5.6	3.8	− 2.1	+ 3.4
417	do	2 gm. dried blood+1 gm. $CaCO_3$	1,130.0	2.2	+1,122.3	+ 1.8
417	do	2 gm. dried blood+1 gm. $CaCO_3$+infusion from No. 416.	1,102.0	10.1	+1,094.3	+ 9.7

Practically no effects were produced by adding infusions from the cultivated to the uncultivated soils, or vice versa, except where dried blood and lime were added also. In these instances the infusions from the uncultivated soil caused a decrease in both nitrification and ammonification, whereas adding infusions from the cultivated soil caused a stimulation in nitrification. The inhibiting agent in the uncultivated soil, therefore, seems to be capable of being transferred in a water solution, although the results are not entirely convincing.

In order to study the effects brought about by sterilization, 100-gram portions were heated in an autoclave for two hours at a pressure of two atmospheres. After cooling, dried blood, calcium carbonate, and infusions from the original soils were added, optimum moisture conditions brought about, and incubated for the usual periods. The ammonia and nitrate that accumulated are shown in the following table:

Ammonification and nitrification after sterilizing in autoclave.

[Parts per million.]

Lab. No.	Previous condition.	Treatment.	Ammonia nitrogen found.	Nitrate nitrogen found.
416	Cultivated	No inoculation	12.5	57.5
416	do	Infusion from No 416	18.0	51.0
416	do	Infusion from No. 416+2 gm. dried blood	35.0	51.0
416	do	Infusion from No. 416+2 gm. dried blood+1 gm. $CaCO_3$	37.1	55.5
416	do	Infusion from No. 417	17.2	52.0
416	do	Infusion from No. 417+2 gm. dried blood	31.5	51.0
416	do	Infusion from No. 417+2 gm. dried blood+1 gm. $CaCO_3$	32.1	51.0
417	Uncultivated	No inoculation	12.2	.6
417	do	Infusion from No. 416	19.0	.8
417	do	Infusion from No. 416+2 gm. dried blood	35.2	.8
417	do	Infusion from No. 416+2 gm. dried blood+1 gm. $CaCO_3$	37.4	1.2
417	do	Infusion from No. 417	18.9	.4
417	do	Infusion from No. 417+2 gm. dried blood	31.5	1.4
417	do	Infusion from No. 417+2 gm. dried blood+1 gm. $CaCO_3$	31.6	1.9

These data show that the ammonifying organisms occurring in the cultivated and uncultivated soils are equally active, and that ammon-

ification took place in the two soils at practically the same rates after sterilization. On the other hand, no nitrification took place in the previously cultivated soil, and only to a very slight extent in that uncultivated. Ammonification took place much less vigorously in these soils after having been sterilized than before, although it should be remembered that in the initial stages of the ammonification a much smaller number of organisms was present than originally occurred in the soil. It is probable, however, in view of the absence of nitrification, and the fact that toxic conditions are known to be brought about by steam heat, that conditions somewhat toxic to ammonification were developed. The point of greatest interest in these results is that by sterilization in the autoclave changes were brought about in the cultivated and uncultivated soils, so that ammonification proceeded subsequently at practically the same rates in each.

In order to study the effects of still higher heating, portions of cultivated and uncultivated soils Nos. 329 and 330 were heated in porcelain dishes over the free flame of a Bunsen burner for a period of 10 hours. After cooling, each was treated with dried blood, calcium carbonate, and an active infusion. At the end of the usual incubation periods the ammonia and nitrate were determined, with the following results:

Ammonification and nitrification after burning.

[Parts per million.]

Lab. No.	Previous condition.	Treatment.	Ammonia nitrogen.	Nitrate nitrogen.
329	Cultivated	Immediately after burning	274.0	26.0
329	do	Active infusion	254.0	30.0
329	do	2 gm. dried blood+1 gm. $CaCO_3$+active infusion	899.0	32.4
330	Uncultivated	Immediately after burning	183.0	18.8
330	do	Active infusion	206.0	13.0
330	do	2 gm. dried blood+1 gm. $CaCO_3$+active infusion	929.0	17.1

In the first place heat caused an initial splitting off of a large amount of ammonia and a partial decomposition of the nitrate.[1] The subsequent ammonification was practically the same in each soil, however, while nitrification took place to a slight extent in soil No. 329 only. Thus, again, it is shown that heat reacts on the cultivated and uncultivated soils of Hawaii in such way as to bring them into similar conditions so far as bacterial action is concerned.

EFFECTS OF PARTIAL STERILIZATION.

For a number of years it has been known that plant stimulation may be brought about in soils by means of heating and by the application of such substances as carbon bisulphid, chloroform, etc. The

[1] Hawaii Sta. Bul. 30 (1913).

effects produced thereby are now commonly considered to be due to effects produced on the soil organisms either directly or indirectly. It has been known for some time, for instance, that, while the numbers of bacteria are generally reduced by partial sterilization, later on the bacterial population rises to abnormal proportions.

The different views held on this subject may be briefly summarized under three heads. First, the stimulation theory, by which it is held that the organisms which survive receive a direct stimulation from the treatment in addition to being supplied with an increase in food, made available by the sterilization, through decomposition of the soil organic matter, and in the cells of the organisms killed by the treatment. Second, the protozoan theory, according to which partial sterilization causes a destruction of certain phagocytes, which are supposed to feed upon the bacteria of soils and thus keep their numbers, and consequently their efficiency, in check. The amœbæ, infusoria, etc., being killed by the treatment, the remaining bacteria then multiply to great numbers, and the greater numbers of bacteria thus arising, rather than increased efficiency, cause the production of greater amounts of available nitrogen. Third, the bacteriotoxin and soil-film theory, according to which soils may contain substances poisonous to bacteria, which substances are capable of being decomposed at the temperatures employed in partial sterilization by means of heat. Volatile antiseptics, on the other hand, bring about bacterial stimulation through the solvent effects exerted on certain organic substances which surround the soil particles and which partially waterproof them, thus protecting the organic substances from the attack of bacteria. Upon evaporating the antiseptic, the dissolved substances become redistributed in such way as to leave the soil particles more open to bacterial invasion.

It will be noted that all but one of the theories above named presuppose the existence of a limiting agent in soils, the presence of one or more factors which operate to hold in check bacterial action. From the experiments above recorded it seems that the uncultivated soils of Hawaii contain some agent which limits bacterial action. It was shown, for instance, that the low bacterial efficiency is not due to the absence of oxygen as such, nor the specific organism, but rather to the presence of some factor which is susceptible of alteration by aeration, but considerable time is required for the aeration to exert its effects. It was suggested, therefore, that the toxic condition might be susceptible of alteration by partial sterilization. For this reason the following experiments were undertaken.

In these experiments the methods employed by Russell and Hutchinson[1] were used. The soils on reaching the laboratory were

[1] Jour. Agr. Sci., 3 (1909), pp. 111–144; also Russell and Golding, ibid., 5 (1912), pp. 27–47; Russell and Petherbridge, 5 (1912), pp. 86–111; Russell and Hutchinson, ibid., 5 (1913), pp. 152–221.

spread out on large sheets of paper and after becoming air dry, different portions were treated as follows: One portion of each soil containing from 600 to 800 grams, was heated in a water oven at 98° C. for two hours, then immediately placed in screw-cap glass jars. Other portions of equal weight were thoroughly mixed with toluol and carbon bisulphid at the rate of 4 cubic centimeters per 100 grams of soil, then placed in tight-fitting screw-cap jars, in which condition the sample stood for three days. These portions were then spread out in thin layers on clean paper, and the antiseptic allowed to evaporate for three additional days, when no odor of the antiseptic could be detected. The treated samples and also an untreated portion of each soil were then brought to optimum moisture by adding sterile water, placed in large fruit jars, loosely stoppered, and kept in a dark closet at about 28° C. The moisture was maintained by the addition of sterile water from time to time. At different intervals portions were withdrawn with a sterile spatula, and the nitrate and ammonia determined. The results are shown in the following table:

Ammonia and nitrate nitrogen in partially sterilized soils.

[Parts per million of the air-dried soil.]

AMMONIA NITROGEN.

Treatment.	Cultivated soil No. 329.					Uncultivated soil No. 330.				
	Before treatment.	After 8 days.	After 14 days.	After 21 days.	After 28 days.	Before treatment.	After 8 days.	After 14 days.	After 21 days.	After 28 days.
Untreated	33.6	39.2	22.4	28.0	33.6	14.0	19.6	11.2	22.4	28.0
Heated to 98° C	33.6	104.8	106.4	128.8	123.2	14.0	67.2	72.8	84.0	78.4
Toluol	33.6	117.6	114.8	126.0	131.6	14.0	89.6	114.8	120.4	126.0

NITRATE NITROGEN.

Untreated	75.0	220.0	220.0	168.0	220.0	1.3	13.0	14.6	18.8	7.0
Heated to 98° C	75.0	150.0	148.0	140.0	164.0	1.3	5.0	6.0	5.0	2.0
Toluol	75.0	190.0	180.0	160.0	65.0	1.3	38.0	34.8	33.2	27.0

TOTAL NITRATE AND AMMONIA NITROGEN.

Untreated	108.6	259.2	242.4	196.0	253.6	15.3	32.6	25.8	41.2	35.0
Heated to 98° C	108.6	254.8	254.4	268.8	287.2	15.3	72.2	78.8	89.0	80.4
Toluol	108.6	307.6	294.8	286.0	196.6	15.3	127.6	149.6	153.6	153.0

GAINS IN NITRATE AND AMMONIA NITROGEN.

Untreated	.....	150.6	133.8	87.4	145.0	.....	17.3	10.5	25.9	19.7
Heated to 98° C	.....	146.2	145.8	160.2	178.6	.....	56.9	63.5	73.7	65.1
Toluol	.....	199.0	186.2	177.4	88.0	.....	112.3	134.3	138.3	137.7

Ammonia and nitrate nitrogen in partially sterilized soils—Continued.

AMMONIA NITROGEN.

Treatment.	Cultivated soil No. 416.					Uncultivated soil No. 417.				
	At the beginning.	After 7 days.	After 14 days.	After 21 days.	After 28 days.	At the beginning.	After 7 days.	After 14 days.	After 21 days.	After 28 days.
Untreated	19.6	11.2	14.0	16.8	28.0	22.4	53.2	42.0	48.8	42.0
Heated to 98° C	33.6	86.8	100.8	106.4	109.2	32.8	112.0	148.4	159.6	168.0
Toluol	22.4	78.4	100.8	109.2	120.4	25.2	120.4	154.0	162.4	179.2

NITRATE NITROGEN.

Untreated	70.0	90.0	92.0	86.0	90.0	0.6	0.6	13.0	8.8	12.0
Heated to 98° C	68.0	65.0	70.0	64.0	60.0	.7	2.3	8.8	.7	.6
Toluol	60.0	62.0	64.0	60.0	60.0	.4	.5	12.0	.7	.6

TOTAL NITRATE AND AMMONIA NITROGEN.

Untreated	89.6	101.2	106.0	102.8	118.0	23.0	53.8	55.0	57.6	54.0
Heated to 98° C	101.6	151.8	170.8	170.4	169.2	33.5	114.3	157.2	160.3	168.6
Toluol	82.4	140.4	164.8	169.2	180.4	25.6	120.9	166.0	163.1	179.8

GAINS IN NITRATE AND AMMONIA NITROGEN.

Untreated	.....	11.6	16.4	13.2	28.4	.....	30.8	32.0	34.6	31.0
Heated to 98° C	.....	50.2	69.2	68.8	67.6	.....	80.8	123.7	126.8	135.1
Toluol	.....	58.0	82.4	86.8	98.0	.....	95.3	140.4	137.5	154.2

The above data show that notable effects were produced by partial sterilization. For instance, as a result of the treatment, the ammonia content increased in both cultivated and uncultivated soils during the entire 28-day period of observation. Nitrification, on the other hand, was totally inhibited in soils Nos. 416 and 417, while in Nos. 329 and 330 it was considerably checked in most instances. The data showing the gains in total ammonia and nitrate bring out the effects more correctly since the nitrate formed must have passed through the ammonia stage. Cultivated soil No. 329 gained 33.6 parts per million as a result of heating, while the uncultivated soil No. 330 gained 45.4 parts. Treatment with toluol affected ammonification in soil No. 329 very much the same as heating, while in No. 330 toluol produced notably greater effects, but in the former instances denitrification became excessive, the nitrate content having decreased, after the eighth day, from 190 parts to 65 parts per million. Some denitrification took place in soil No. 330, although to a much less extent.

Considering soils Nos. 416 and 417, we find that partial sterilization produced similar effects in both the cultivated and uncultivated soils, causing, on the one hand, a marked stimulation in the ammonification and, on the other, totally preventing nitrification. It is also noteworthy that at the end of 28 days the total nitrate and ammonia

nitrogen in the treated portions of the cultivated and uncultivated soils was practically the same. Ammonification was therefore the more markedly stimulated in the uncultivated soil, since the available nitrogen originally present was considerably less than in the cultivated soil.

In order to determine whether effects similar to those observed above would be produced in other island soils, the same treatments were applied to a soil from the experiment station grounds, No. 288, and to a rice soil, No. 292, which was previously devoted to rice experiments by this station. The results follow:

Effects of partial sterilization.

[Parts per million.]

AMMONIA NITROGEN.

Treatment.	Soil No. 288.					Soil No. 292.					
	Before treatment.	After 8 days.	After 14 days.	After 21 days.	After 28 days.	Before treatment.	After 7 days.	After 14 days.	After 21 days.	After 28 days.	After 35 days.
Untreated	16.8	28.0	11.2	16.4	14.0	2.0	19.6	14.0	11.2	11.2	14.0
Heated to 98° C.	16.8	44.8	39.2	39.2	22.4	2.0	33.6	39.2	36.4	42.0	47.6
Toluol	16.8	36.4	11.2	14.0	19.6	2.0	30.8	30.8	16.8	11.2	16.8
NITRATE NITROGEN.											
Untreated	4.7	36.0	44.0	40.0	40.0	0.5	24.0	27.5	33.4	37.6	47.0
Heated to 98° C.	4.7	28.0	37.2	42.0	67.0	.5	6.5	14.5	16.0	24.8	26.5
Toluol	4.7	32.0	68.0	[1] 39.0	70.0	.5	1.4	14.5	25.0	38.0	47.0
TOTAL AMMONIA AND NITRATE NITROGEN.											
Untreated	21.5	64.0	55.2	56.4	54.0	2.5	43.6	41.5	44.6	48.8	61.0
Heated to 98° C.	21.5	72.8	76.4	81.2	89.4	2.5	40.1	53.7	52.4	66.8	74.1
Toluol	21.5	68.4	79.2	53.0	89.6	2.5	32.2	45.3	41.8	49.2	63.8
GAINS IN AMMONIA AND NITRATE NITROGEN.											
Untreated	.....	42.5	33.7	34.9	32.5	41.1	.....	39.0	42.1	46.3	58.5
Heated to 98° C.	.....	51.3	54.9	59.7	67.9	37.6	.....	51.2	49.9	64.3	71.6
Toluol	.....	46.9	57.7	31.5	68.1	29.7	.....	42.8	39.3	46.7	61.3

[1] Too low, probably due to error of determination.

Thus it is shown that ammonification was greatly stimulated in soil No. 288 by heating to 98° C. and by the addition of toluol. But the ammonia was prevented from accumulating toward the close of the experimental period by the activity of nitrification, whereas nitrification was partially inhibited in soil No. 292. The total ammonia and nitrate present at the different intervals show that an increase in the amounts of available nitrogen was produced by partial sterilization, but the effectiveness of the treatment was much greater in the soil from the experiment station grounds than in the rice soil. In fact, the total ammonia and nitrate at the different intervals in the portions of soil No. 292 treated with toluol were practically the

same as those in the untreated portions, while the increases in the heated portions were small. The effects produced with soil No. 288, on the other hand, were notable, amounting to more than 100 per cent increases in the available nitrogen.

The conclusion to be drawn from the above experiments is that ammonification in Hawaiian soils may be greatly stimulated by partial sterilization, and that, in a few instances, stimulation may result in nitrification, although it is temporarily inhibited.

It is claimed by Russell and Hutchinson [1] that the stimulation given to ammonification by partial sterilization may be slowly overcome by reinoculation with a small portion of the original soil. They found, for example, that the numbers of bacteria in the reinoculated portions decreased, gradually diminishing in numbers until approximately the same numbers were found as in the untreated soil, and that the ammonia content also decreased, the amounts found being roughly proportional to the number of bacteria present. They attribute these phenomena to the reintroduction into the treated soil of the limiting agent (believed by them to be protozoa) that occurs in natural soils, which agent, they hold, is destroyed by partial sterilization. For the purpose of studying the effects thus produced in Hawaiian soils, the same treatments as were employed in the previous experiments were applied to different soils, and they were reinoculated by adding 5 per cent by weight of the original soil. Observations over a much longer period than was employed in the previous experiments were made and optimum moisture conditions maintained throughout. The results are recorded in the following tables:

Effects of partial sterilization, soil No. 428.

[Parts per million.]

AMMONIA NITROGEN.

Treatment.	At the beginning.	After 7 days.	After 14 days.	After 21 days.	After 35 days.	After 63 days.	After 138 days.	After 201 days.
Untreated	106.4	123.2	123.2	95.2	75.6	5.6	5.6	14.0
Heated to 98° C	103.6	128.8	141.7	159.6	171.6	207.2	159.6	11.2
Heated + 5 per cent original soil	103.4	137.2	145.6	154.0	162.4	16.8	2.8	16.4
Toluol 4 per cent	100.8	151.2	154.0	170.8	182.0	210.0	120.4	22.4
Toluol + 5 per cent original soil	100.8	154.0	148.4	170.8	171.6	14.0	5.6	14.0
CS_2 4 per cent	98.0	126.0	142.8	156.4	164.0	210.0	240.8	268.8
CS_2 + 5 per cent original soil	98.0	137.2	145.6	168.0	164.0	204.4	168.0	11.2

NITRATE NITROGEN.

Untreated	55.5	74.0	68.0	88.0	94.0	225.0	310.0	330.0
Heated to 98° C	53.5	64.0	67.5	58.0	56.0	77.5	160.0	280.0
Heated + 5 per cent original soil	53.5	64.0	62.0	56.0	60.0	235.0	380.0	340.0
Toluol 4 per cent	54.5	64.0	62.0	56.0	60.0	75.0	180.0	260.0
Toluol + 5 per cent original soil	54.5	64.0	62.0	60.0	60.0	232.5	290.0	330.0
CS_2 4 per cent	55.0	56.0	64.0	64.0	56.0	72.0	80.0	75.0
CS_2 + 5 per cent original soil	55.0	60.0	62.0	64.0	62.0	67.5	185.0	290.0

[1] Loc. cit.

Effects of partial sterilization, soil No. 428—Continued.

TOTAL NITRATE AND AMMONIA NITROGEN.

Treatment.	At the beginning.	After 7 days.	After 14 days.	After 21 days.	After 35 days.	After 63 days.	After 138 days.	After 201 days.
Untreated	161.9	197.2	191.2	183.2	169.6	230.6	315.6	344.0
Heated to 98° C	157.1	192.8	209.2	217.6	227.6	284.8	319.6	291.2
Heated + 5 per cent original soil	156.9	201.2	207.6	210.0	222.4	251.8	382.8	356.4
Toluol 4 per cent	155.3	215.2	216.0	226.8	242.0	285.0	300.4	282.4
Toluol + 5 per cent original soil	155.3	218.0	210.4	230.8	231.6	246.5	295.6	344.0
CS_2 4 per cent	153.0	182.0	206.8	220.4	220.0	282.0	320.8	343.8
CS_2 + 5 per cent original soil	153.0	197.2	207.6	232.0	226.0	271.9	353.0	301.2

GAINS IN NITRATE AND AMMONIA NITROGEN.

Untreated		35.3	29.3	21.3	7.7	68.7	153.7	182.1
Heated to 98° C		35.7	52.1	60.5	70.5	127.7	162.5	134.1
Heated + 5 per cent original soil		44.3	50.7	53.1	65.5	94.9	225.9	199.5
Toluol 4 per cent		59.9	60.7	71.5	86.7	129.7	145.1	127.1
Toluol + 5 per cent original soil		62.7	55.1	75.5	76.3	91.2	140.3	188.7
CS_2 4 per cent		29.0	53.8	67.4	67.0	129.0	167.8	190.8
CS_2 + 5 per cent original soil		44.2	54.6	79.0	73.0	118.9	200.0	148.2

Effects of partial sterilization, soil No. 485.

[Parts per million.]

AMMONIA NITROGEN.

Treatment.	Before treatment.	After 7 days.	After 14 days.	After 21 days.	After 28 days.	After 35 days.	After 94 days.	After 156 days.
Untreated	7.0	8.4	16.8	14.0	22.4	8.4	8.4	14.0
Heated to 98° C	7.0	36.4	56.0	46.7	22.4	16.8	5.6	11.2
Heated + 5 per cent original soil	7.0	39.2	44.8	39.2	8.4	11.2	2.8	14.0
Toluol 4 per cent	7.0	47.6	61.6	61.6	33.6	11.2	8.4	11.2
Toluol + 5 per cent original soil	7.0	42.0	56.0	33.6	14.0	11.2	8.4	14.0
CS_2 4 per cent	7.0	44.8	64.4	67.2	70.0	70.0	86.8	75.6
CS_2 + 5 per cent original soil	7.0	42.0	61.6	67.2	70.0	72.8	11.2	11.2

NITRATE NITROGEN.

Untreated	10.0	18.0	23.5	30.0	30.0	32.0	62.5	87.5
Heated to 98° C	10.0	13.0	15.0	30.0	66.0	82.5	92.5	120.0
Heated + 5 per cent original soil	10.0	14.8	20.0	32.5	70.0	75.0	92.5	117.5
Toluol 4 per cent	10.0	10.0	8.4	16.0	36.0	65.0	80.0	100.0
Toluol + 5 per cent original soil	10.0	12.0	14.8	27.5	62.0	72.5	97.5	105.0
CS_2 4 per cent	10.0	1.0	2.8	5.7	7.6	8.0	10.6	31.5
CS_2 + 5 per cent original soil	10.0	2.5	5.0	8.5	8.6	9.8	97.5	83.5

TOTAL NITRATE AND AMMONIA NITROGEN.

Untreated	17.0	26.4	40.3	44.0	52.4	40.4	70.9	101.5
Heated to 98° C	17.0	49.4	71.0	76.7	88.4	99.3	98.1	131.2
Heated + 5 per cent original soil	17.0	54.0	64.8	71.7	78.4	86.2	95.3	131.5
Toluol 4 per cent	17.0	57.6	70.0	77.6	69.6	76.2	88.4	111.2
Toluol + 5 per cent original soil	17.0	54.0	70.8	61.1	76.0	83.7	105.9	119.0
CS_2 4 per cent	17.0	45.8	67.2	72.9	77.6	78.0	97.4	107.1
CS_2 + 5 per cent original soil	17.0	44.5	66.6	75.7	78.6	82.6	108.7	94.7

GAINS IN NITRATE AND AMMONIA NITROGEN.

Untreated		9.4	23.3	27.0	35.4	23.4	53.9	84.5
Heated to 98° C		32.4	54.0	59.7	71.4	82.3	81.1	114.2
Heated + 5 per cent original soil		37.0	47.8	54.7	61.4	69.2	78.3	114.5
Toluol 4 per cent		40.6	53.0	60.6	52.6	59.2	71.4	94.2
Toluol + 5 per cent original soil		37.0	53.8	44.1	59.0	66.7	88.9	102.0
CS_2 4 per cent		28.8	50.2	55.9	60.6	61.0	80.4	90.1
CS_2 + 5 per cent original soil		27.5	49.6	58.7	61.6	65.6	91.7	77.7

Effects of partial sterilization, soil No. 486.

[Parts per million.]

AMMONIA NITROGEN.

Treatment.	Before treatment.	After 7 days.	After 14 days.	After 21 days.	After 28 days.	After 35 days.	After 94 days.	After 156 days.
Untreated	8.0	8.4	14.0	14.0	11.2	14.0	16.8	11.2
Heated to 98° C	8.0	72.8	100.8	84.0	39.2	16.8	8.4	14.0
Heated + 5 per cent original soil	8.0	70.0	81.2	72.8	16.8	16.8	16.8	14.0
Toluol 4 per cent	8.0	78.4	98.0	103.6	106.4	117.6	8.4	11.2
Toluol + 5 per cent original soil	8.0	70.0	89.6	100.8	50.4	16.8	11.2	14.0
CS_2 4 per cent	8.0	64.6	89.6	95.2	95.2	100.8	114.8	112.0
CS_2 + 5 per cent original soil	8.0	72.8	100.8	95.2	106.2	109.2	11.2	11.2

NITRATE NITROGEN.

Untreated	6.0	18.0	20.0	25.0	25.0	34.0	70.0	87.5
Heated to 98° C	6.0	14.0	10.0	19.0	55.0	102.5	107.5	170.0
Heated + 5 per cent original soil	6.0	8.0	14.0	26.0	64.0	90.0	117.5	140.0
Toluol 4 per cent	6.0	5.0	5.8	5.5	5.5	7.5	90.0	140.0
Toluol + 5 per cent original soil	6.0	5.0	7.0	9.0	40.0	91.0	120.0	135.0
CS_2 4 per cent	6.0	0.5	2.0	2.5	3.0	3.0	7.6	22.0
CS_2 + 5 per cent original soil	6.0	0.5	2.0	3.0	3.0	2.8	102.5	130.0

TOTAL NITRATE AND AMMONIA NITROGEN.

Untreated	14.0	26.4	34.0	39.0	36.2	48.0	86.8	98.7
Heated to 98° C	14.0	86.8	110.8	103.0	94.2	119.3	115.9	184.0
Heated + 5 per cent original soil	14.0	78.0	95.2	98.9	80.8	106.8	134.3	154.0
Toluol 4 per cent	14.0	83.4	103.8	109.1	111.9	125.1	98.4	151.2
Toluol + 5 per cent original soil	14.0	75.0	96.6	109.8	90.4	107.8	131.2	149.0
CS_2 4 per cent	14.0	65.1	91.6	97.7	98.2	103.8	122.4	134.0
CS_2 + 5 per cent original soil	14.0	73.3	102.8	98.2	109.4	112.0	113.7	141.2

GAINS IN NITRATE AND AMMONIA NITROGEN.

Untreated	.....	12.4	20.0	25.0	22.2	34.0	72.8	84.7
Heated to 98° C	.....	72.8	96.8	89.0	80.2	105.3	101.9	170.0
Heated + 5 per cent original soil	.....	64.0	81.2	84.9	66.8	92.8	120.3	140.0
Toluol 4 per cent	.....	69.4	89.8	95.1	97.9	111.1	84.4	137.2
Toluol + 5 per cent original soil	.....	61.0	82.6	95.8	76.4	93.8	117.2	135.0
CS_2 4 per cent	.....	51.1	77.6	83.7	84.2	89.8	108.4	120.0
CS_2 + 5 per cent original soil	.....	59.3	88.8	84.2	95.4	98.0	99.7	127.2

It will be seen that in each soil an increase in the ammonia content was effected by partial sterilization, but that after the lapse of a certain interval of time, varying in the different soils studied, and also in the same soil when partially sterilized by different means, nitrification set in, with the result that the ammonia content became reduced to a low and practically equal concentration in all the different portions of each soil, with the exception of those treated with carbon bisulphid. In this case the ammonia content increased throughout the time of observation, only slight nitrification having taken place, and then only after a lapse of several months. The addition of 5 per cent of the original soil to the partially sterilized portions produced more vigorous nitrification in the early periods, due no doubt to the introduction of active nitrifying organisms. The method of effecting partial sterilization probably killed the greater numbers of the nitrifying organisms present, as has been shown to take place by Russell and Hutchinson and others.

The total ammonia and nitrate present is of especial interest. It is noteworthy that in soil No. 428 partial sterilization produced a considerable increase in the available nitrogen at the different intervals up to 63 days from the time of treatment. After this time the available nitrogen continued to accumulate up to the 138th day, but at rates correspondingly less in the treated than in the untreated portions. Consequently the gains in available nitrogen during this period were less than during earlier periods. From the 138th to the 201st day, most of the partially sterilized portions lost available nitrogen, whereas the accumulation continued in the untreated portions; consequently at the end of the experimental period the untreated portions contained more nitrate and ammonia than a number of treated portions.

It will also be noted that reinoculation with 5 per cent of the original soil of the portions heated and treated with toluol caused an increase in available nitrogen, but in the carbon bisulphid portions exactly opposite effects were produced, that is, reinoculation resulted in a notable decrease in the available nitrogen.

Turning to soils Nos. 485 and 486, it will be seen that the partial sterilization stimulated ammonification throughout the experiment. Reinoculating the portions of No. 485, heated and treated with toluol, and the portions of No. 486, treated with toluol and carbon bisulphid, on the whole produced no effects, while the reinoculation of No. 485, treated with carbon bisulphid, and the heated portion of No. 486 caused a considerable reduction in the total nitrate and ammonia. On the whole, then, the effects produced by reinoculating the partially sterilized soils are not in harmony with those found by Russell et al.

It has been shown by Gainey [1] that the use of small amounts of antiseptics results in immediate stimulation of the bacteria without a reduction in the numbers present, such as takes place where larger amounts are used. Gainey further found that the application of different volatile antiseptics produced notable stimulation in the growth of crops, but he failed to detect a corresponding effect on the numbers of bacteria present.[2]

In the experiments reported above, the antiseptic was allowed to evaporate from the soil until no further odor could be detected. The treatments were made on air-dried soils, but, upon bringing to optimum moisture content and allowing to stand a few days, a faint odor of the antiseptics was detected in most instances. Where carbon bisulphid was employed rather distinct odors of the substance were noticed till near the close of the period of observation. Since it has

[1] Missouri Bot. Gard., Ann. Rpt., 23 (1912), pp. 147–169.

[2] In Gainey's experiments the moisture content of the soil was brought to one-third or one-half saturation before the antiseptic was added, and since the substances used are miscible with water to a slight extent only, it is possible that the different organisms present did not come in contact with the antiseptics.

been shown that Hawaiian soils have a remarkably high absorptive capacity [1] it was suggested that the treated portions absorbed small amounts of the antiseptics, which exerted stimulative effects on the surviving bacteria. As shown in the preceding tables, where carbon bisulphid was employed, nitrification did not set in until after a much longer time than when other methods of effecting partial sterilization were used. This may have been due to the inhibiting action of the carbon bisulphid absorbed.

The soils employed in the following experiment were Nos. 288 and 329, the same as employed previously, each of which had been found to show marked effects from partial sterilization. The portions used in previous experiments were treated soon after becoming air dry. In the following experiments, however, the soils had remained in the laboratory in the air-dried state for several months previous to the time of treatment. In the following table are shown the results:

Effects of partial sterilization on thoroughly desiccated soils.

[Parts per million.]

AMMONIA NITROGEN.

Treatment.	Soil No. 288.				Soil No. 329.			
	At the beginning.	After 15 days.	After 33 days.	After 82 days.	At the beginning.	After 15 days.	After 33 days.	After 80 days.
Untreated	19.6	22.4	5.6	2.8	56.0	72.8	89.6	50.4
Heated to 98° C	19.6	25.2	44.8	5.6	61.6	72.8	103.6	128.8
Heated+5 per cent original soil	19.6	16.2	25.2	5.6	61.6	72.8	95.2	140.0
Toluol 0.2 per cent	16.2	33.6	44.8	5.6	61.6	50.4	84.0	131.6
Toluol 4 per cent	19.6	00.0	8.4	8.4	64.4	64.4	75.6	100.8
Toluol 4 per cent+5 per cent original soil	19.6	00.0	5.6	5.6	64.4	72.8	92.4	109.2
CS_2 0.2 per cent	19.6	36.4	58.8	89.6	70.0	103.6	84.0	126.0
CS_2 4 per cent	16.2	42.0	70.0	84.0	70.0	61.6	84.0	162.4
CS_2 4 per cent+5 per cent original soil	16.2	39.2	58.8	72.8	70.0	75.6	92.4	162.4

NITRATE NITROGEN.

Untreated	17.5	41.0	50.0	120.0	152.0	137.5	195.0	280.0
Heated to 98° C	16.0	51.0	52.5	115.0	164.0	137.5	185.0	250.0
Heated to 98° C.+5 per cent original soil	16.0	28.5	52.0	115.0	164.0	145.0	195.0	205.0
Toluol 0.2 per cent	18.0	21.0	36.0	110.0	160.0	124.0	180.0	220.0
Toluol 4 per cent	16.8	46.0	55.0	110.0	160.0	150.0	195.0	225.0
Toluol 4 per cent+5 per cent original soil	16.8	35.0	60.0	120.0	160.0	137.5	185.0	200.0
CS_2 0.2 per cent	10.0	12.5	20.0	48.0	130.0	145.0	185.0	180.0
CS_2 4 per cent	4.5	1.0	5.5	35.0	160.0	147.0	175.0	130.0
CS_2 4 per cent+5 per cent original soil	4.5	1.0	5.0	3.0	160.0	132.5	170.0	135.0

TOTAL NITRATE AND AMMONIA NITROGEN.

Untreated	37.1	63.4	55.6	122.8	208.0	210.3	284.6	330.4
Heated to 98° C	35.6	76.2	97.3	120.6	225.6	210.3	288.6	378.8
Heated to 98° C.+5 per cent original soil	35.6	44.7	77.2	120.6	225.6	217.8	290.2	345.0
Toluol 0.2 per cent	34.2	54.6	80.8	115.6	221.6	174.4	264.0	351.6
Toluol 4 per cent	36.4	46.0	63.4	118.4	224.4	214.4	270.6	325.8
Toluol 4 per cent+5 per cent original soil	36.4	35.0	65.6	125.6	224.4	210.3	277.4	309.2
CS_2 0.2 per cent	29.6	48.9	78.8	137.6	200.0	248.6	269.0	306.0
CS_2 4 per cent	20.7	43.0	75.5	119.0	230.0	208.6	259.0	292.4
CS_2 4 per cent+5 per cent original soil	20.7	40.2	63.8	75.8	230.0	208.1	262.4	297.4

[1] Hawaii Sta. Bul. 35.

Effects of partial sterilization on thoroughly desiccated soils—Continued.

GAIN (+) OR LOSS (−) IN NITRATE AND AMMONIA NITROGEN.

Treatment.	Soil No. 288.				Soil No. 329.			
	At the beginning.	After 15 days.	After 33 days.	After 82 days.	At the beginning.	After 15 days.	After 33 days.	After 80 days.
Untreated	.....	+26.3	+18.5	+ 85.7	.....	+ 2.3	+76.6	+122.4
Heated to 98° C	.....	+40.6	+61.7	+ 85.0	.....	−15.3	+63.0	+153.2
Heated to 98° C.+5 per cent original soil	.....	+ 9.1	+41.6	+ 85.0	.....	− 7.8	+64.6	+119.4
Toluol 0.2 per cent	.....	+20.4	+46.6	+ 81.4	.....	−47.2	+42.4	+130.0
Toluol 4 per cent	.....	+ 9.6	+27.0	+ 82.0	.....	−10.0	+46.2	+101.4
Toluol 4 per cent+5 per cent original soil	.....	− 1.4	+29.2	+ 89.2	.....	−14.1	+53.0	+ 84.8
CS_2 0.2 per cent	.....	+19.3	+49.2	+108.0	.....	+48.6	+69.0	+106.0
CS_2 4 per cent	.....	+22.3	+54.8	+ 98.3	.....	−21.4	+29.0	+ 62.4
CS_2 4 per cent+5 per cent original soil	.....	+19.5	+43.1	+ 55.1	.....	−21.9	+32.4	+ 67.4

The above data show that greater irregularity resulted from the treatments than in any of the previously recorded experiments. At the end of 15 days no important increase in available nitrogen was found, except in the heated portions of soil No. 288 and the portions of No. 329 treated with 0.2 per cent carbon bisulphid. On the other hand, a decrease was observed in a number of instances. At that time the portions of No. 288 treated with carbon bisulphid contained practically no nitrate. After 33 days each of the treated portions contained an increased amount of available nitrogen, whereas no stimulation was manifest in soil No. 329, and after 82 days no increase was found in any instance except the portion of No. 288 treated with 0.2 per cent carbon bisulphid and those of No. 329 heated and treated with toluol. Reinoculating the portions of No. 288 treated with toluol was without effect, whereas in No. 329 it caused a reduction of from 325.8 to 309.2 parts per million. The use of 0.2 per cent of both toluol and carbon bisulphid proved equally as effective as 4 per cent.

It is notable that irregular and sometimes negative effects were produced by partial sterilization when applied after the soils had been air dry for several months, while the same treatment applied to the fresh soils produced regular and stimulating effects.

Before taking up the general discussion of the foregoing results, it will be of interest to examine the data already submitted, with a view to determining how long the stimulation continued in the different soils studied. In the table following the data presented in the preceding tables are brought together for the purpose of showing the gains in available nitrogen during the different periods.

Gain (+) or loss (−) in ammonia and nitrate nitrogen during successive periods.

[Parts per million.]

Treatment.	Soil No. 329.						Soil No. 330.					
	Days 1–8.	Days 8–14.	Days 14–21.	Days 21–28.	Days 8–28.	Days 14–28.	Days 1–8.	Days 8–14.	Days 14–21.	Days 21–28.	Days 8–28.	Days 14–28.
Check	+150.6	−16.8	−46.4	+57.6	− 5.6	+11.2	+ 17.3	− 6.8	+15.4	− 6.2	+ 2.4	+ 9.2
Heated	+146.2	− .4	+14.4	+18.4	+ 32.4	+32.8	+ 56.9	+ 6.6	+10.2	− 8.6	+ 8.2	+ 1.6
Toluol	+199.0	−12.8	− 8.8	−89.4	−111.0	−98.2	+112.[illegible]	+22.0	+ 4.0	− .6	+25.4	+ 3.4

Treatment.	Soil No. 416.						Soil No. 417.					
	Days 1–7.	Days 7–14.	Days 14–21.	Days 21–28.	Days 7–28.	Days 14–28.	Days 1–7.	Days 7–14.	Days 14–21.	Days 21–28.	Days 7–28.	Days 14–28.
Check	+11.6	+ 4.8	− 3.2	+15.2	+16.8	+12.0	+ 30.8	+ 1.2	+ 2.6	− 3.6	+ 0.2	− 1.0
Heated	+50.2	+19.0	− .4	− 1.2	+17.4	− 1.6	+ 80.8	+42.9	+ 3.1	+ 8.3	+54.3	+11.4
Toluol	+58.0	+24.4	+ 4.4	+11.2	+40.0	+15.6	+ 95.3	+45.1	− 2.9	+16.7	+58.9	+13.8

Treatment.	Soil No. 428.								
	Days 1–7.	Days 7–14.	Days 14–21.	Days 21–35.	Days 35–63.	Days 63–138.	Days 138–201.	Days 7–201.	Days 14–201.
Check	+35.3	− 6.0	− 8.0	−13.6	+61.0	+ 85.0	+28.4	+146.8	+152.8
Heated	+35.7	+16.4	+ 8.4	+10.0	+57.2	+ 34.8	−28.4	+ 98.4	+ 82.0
Heated+5 per cent original soil	+44.3	+ 6.4	+ 2.4	+12.4	+29.4	+131.0	−26.4	+155.2	+148.8
Toluol	+59.9	+ .8	+10.8	+15.2	+43.0	+ 15.4	−18.0	+ 67.2	+ 66.4
Toluol+5 per cent original soil	+62.7	− 7.6	+20.4	+ .8	+14.9	+ 49.1	+48.4	+126.0	+133.6
CS_2	+29.0	+24.8	+13.6	− .4	+62.0	+ 38.8	+23.0	+161.8	+137.0
CS_2+5 per cent original soil	+44.2	+10.4	+24.4	− 6.0	+45.9	+ 81.1	−51.8	+104.0	+ 93.6

Treatment.	Soil No. 485.								
	Days 1–7.	Days 7–14.	Days 14–21.	Days 21–28.	Days 28–35.	Days 35–94.	Days 94–156.	Days 7–156.	Days 14–156.
Check	+ 9.4	+13.9	+3.7	+ 8.4	−12.0	+30.5	+30.6	+75.1	+61.2
Heated	+32.4	+21.6	+5.7	+11.7	+10.9	− 1.2	+33.1	+81.8	+60.2
Heated+5 per cent original soil	+37.0	+10.8	+6.9	+ 6.7	+ 7.8	+ 9.1	+36.2	+77.5	+66.7
Toluol	+40.6	+12.4	+7.6	− 8.0	+ 6.6	+12.2	+22.8	+53.6	+41.2
Toluol+5 per cent original soil	+37.0	+16.8	−9.7	+14.9	+ 7.7	+22.2	+13.1	+65.0	+48.2
CS_2	+28.8	+21.4	+5.7	+ 4.7	+ .4	+19.4	+ 9.7	+61.3	+39.9
CS_2+5 per cent original soil	+27.5	+22.1	+9.1	+ 2.9	+ 4.0	+26.1	−14.0	+50.2	+28.1

Treatment.	Soil No. 486.								
	Days 1–7.	Days 7–14.	Days 14–21.	Days 21–28.	Days 28–35.	Days 35–94.	Days 94–156.	Days 7–156.	Days 14–156.
Check	+12.4	+ 7.6	+ 5.0	+ 2.8	+11.8	+38.8	+11.9	+77.9	+70.3
Heated	+72.8	+24.0	− 7.8	− 8.8	+25.1	− 3.4	+68.1	+97.2	+73.2
Heated+5 per cent original soil	+64.0	+17.2	+ 3.7	−18.1	+26.0	+27.5	+19.7	+76.0	+58.8
Toluol	+69.4	+20.4	+ 5.3	+ 2.8	+13.2	−26.7	+52.8	+67.8	+47.4
Toluol+5 per cent original soil	+61.0	+21.6	+13.2	−19.4	+17.4	+23.4	+17.8	+74.0	+52.4
CS_2	+51.1	+26.5	+ 6.1	+ .5	+ 5.6	+18.6	+11.6	+68.9	+42.4
CS_2+5 per cent original soil	+59.3	+29.5	− 4.6	+11.2	+ 2.6	+ 1.7	+27.5	+67.9	+38.4

Gain (+) or loss (−) in ammonia and nitrate nitrogen during successive periods—Contd.

Treatment.	Soil No. 288.				Soil No. 329.[1]			
	Days 1–15.	Days 15–33.	Days 33–82.	Days 15–82.	Days 1–15.	Days 15–33.	Days 33–80.	Days 15–80.
Check	+26.3	− 7.8	+67.2	+59.4	+ 2.3	+74.3	+45.8	+120.1
Heated	+40.6	+21.1	+23.3	+44.4	−15.3	+78.3	+90.2	+168.5
Heated+5 per cent original soil	+ 9.1	+32.5	+43.4	+75.9	− 7.8	+72.4	+54.8	+127.2
Toluol 0.2 per cent	+20.4	+26.2	+34.8	+61.0	−47.2	+89.6	+87.6	+177.2
Toluol 4 per cent	+ 9.6	+17.4	+55.0	+72.4	−10.0	+56.2	+55.2	+111.4
Toluol 4 per cent+5 per cent original soil	− 1.4	+30.6	+60.0	+90.6	−14.1	+67.1	+31.8	+ 98.9
CS_2 0.2 per cent	+19.3	+29.9	+58.8	+88.7	+48.6	+20.4	+37.0	+ 57.4
CS_2 4 per cent	+22.3	+32.5	+43.5	+76.0	−21.4	+50.4	+33.4	+ 83.4
CS_2 4 per cent+5 per cent original soil	+19.5	+23.6	+12.0	+35.6	−21.9	+54.3	+35.0	+ 89.3

[1] Second series.

It is thus shown that while stimulation in ammonification took place in practically every instance, the effects were, with but few exceptions, of short duration, generally ceasing after about 15 days from the time of treatment. Later on, the time varying in the different soils studied, the accumulation of available nitrogen became much slower in the partially sterilized soils, with the result that after a time the effects of the treatment disappeared entirely.

DISCUSSION.

From the investigations above recorded it has been shown that nitrification does not take place in most Hawaiian soils unless tillage is employed, and that the effects produced by aeration may be soon destroyed by continued wet weather. The virgin soils will not support nitrification until they have undergone aeration for several months, while the cultivated soils sustain active nitrification. The lack of nitrification in the former is not due to the absence of nitrifying organisms or acidity. Neither will the mere bringing about of aerobic conditions suffice. It is necessary that oxidizing conditions be maintained for a considerable length of time before nitrification will take place. Hawaiian soils, therefore, require the operation of the weathering process in order to become suitable to the activity of nitrifying bacteria.

Some of the inert virgin soils appear to contain soluble substances which inhibit nitrification. Sterilization in the autoclave affected both cultivated and uncultivated soil in such way as to render them practically equal in regard to subsequent ammonification and brought about conditions toxic to nitrification in each instance; similar effects were produced by heating to still higher temperatures.

Partial sterilization greatly stimulated ammonification, which stimulation persisted usually for about two weeks only, followed then by a retardation in ammonification to a point below that which took place in the untreated soil.

Nitrification was prevented for a short time by partial sterilization, but later regained its activity, finally becoming more active than in the untreated soil. Partial sterilization, however, did not bring about conditions in the inert soils as favorable to nitrification after reinoculation as are produced by continued aeration, and the total available nitrogen found in the partially sterilized soils after a lapse of several months was in a number of instances less than that in the untreated soil.

The reinoculation of partially sterilized soils with 5 per cent of the original soil in some instances caused a temporary reduction in the amount of nitrate and ammonia present, but this effect was not always permanent. In fact, the total nitrate and ammonia, in the soils kept under observation for the greatest length of time, was in some instances increased by reinoculation. In other instances no effects were produced, while in still other instances a permanent reduction in the amounts of available nitrogen was brought about.

The evidence presented above seems to point to the probability that the weathering process, aeration, brings about effects similar in nature but differing in degree from those produced by partial sterilization. These effects are believed by the writer to be in part of the nature of oxidation, but more largely physical, being affected through the changes produced in the colloidal soil films.

The protozoan theory of Russell and Hutchinson appears to be of doubtful application to these soils. It may be stated that some of the soils studied, especially No. 428, contained numerous organisms, apparently infusoria and amœbæ, so numerous indeed as to be easily detected under the low-power microscope.[1] No attempt was made to identify these organisms, but they appeared to be as numerous in the soil treated with toluol and carbon bisulphid [2] some weeks after treatment as in the untreated soil. In the heated portions, however, these organisms were not found, but ammonification was stimulated by the toluol and carbon bisulphid to practically the same extent as by heat.

There is much reason for the belief that the effects produced by different methods of partial sterilization are complicated and can not be satisfactorily explained as being due to a simple cause. It has been repeatedly shown that heating to 98° C. causes more or less decomposition of the organic matter of soils. Such changes certainly affect subsequent bacterial action. Frequently heat has been shown to bring about conditions temporarily toxic to the nitri-

[1] Peck has previously reported the presence of protozoa in Hawaiian soils. See Hawaiian Sugar Planters, Sta., Agr. and Chem. Bul. 34 (1910).

[2] Greig-Smith found that the addition of protozoa to cultures did not reduce the numbers of bacteria during 70 days. Likewise the addition of untreated to partially sterilized soil produced no effects. See Proc. Linn. Soc. N. S. Wales, 37 (1912), pp. 655–672.

fying bacteria. From an extensive investigation carried out in this laboratory it was shown that an increase in the solubility of the inorganic constituents takes place by allowing arable soils to dry out in the laboratory [1] and that a considerably greater increase in solubility was produced by heating to 100° C. These effects, it is believed, are due to alterations in the colloidal films which surround soil particles and which seem to form an especially important feature of Hawaiian soils.

Alterations in the physical nature of colloidal films may reasonably be believed to take place, as a result of drying out or heating, being brought about through dehydration, evaporation from the interior to the exterior of the film, with the consequent deposition at the surface of the film of substances held in solution, and changes in the physical nature of the colloids. Such effects may be conceived to be of considerable biological significance, for new points of attack would thus become exposed, fresh supplies of organic material previously more or less protected from bacterial invasion would be laid open, and an increased food supply brought within their easy reach.

In addition bacteriotoxins, if present, would probably undergo some decomposition, and the organisms surviving the heat would find in the cells of the organisms killed an additional store of material perhaps easily susceptible to decomposition.

The action of volatile antiseptics may be explained on very similar grounds, the effects produced in this case being on soil films, but brought about through solvent effects, after the manner described by Greig-Smith.[2] That there are substances in soils soluble in toluol, carbon bisulphid, chloroform,[3] etc., can hardly be doubted and that such substances would tend to accumulate around soil particles in and on the films also seems very probable. The volatile antiseptics would dissolve some of this material, although the amounts employed be small and upon evaporation a redistribution of the dissolved substances would be expected. Thus new surfaces of organic matter previously protected in part against bacterial invasion would become exposed. It seems probable, moreover, that some direct stimulation would result to the surviving organisms.

Thus, according to this view, the effects produced by partial sterilization are explainable largely on the basis of its making available to the surviving organisms food and organic materials through alterations in the colloidal films. The effects produced by aeration are probably in considerable part of the same nature with the addi-

[1] Hawaii Sta. Bul. 30 (1913).

[2] Proc. Linn. Soc. N. S. Wales, 35 (1910), pp. 808–822B; 36 (1911), pp. 609–612, 679–699; 37 (1912), pp. 238–243, 655–672.

[3] Texas Sta. Bul. 155 (1913).

tion of granulation effects and oxidative [1] decompositions, the latter of which are probably of special importance to the nitrifying organisms.

In these investigations only one of the different methods employed in soil bacteriology, namely, that of measuring the products formed, has been employed. There is urgent necessity for further work on this subject before the fundamental principles can be positively established.

THE LIME-MAGNESIA RATIO.

As stated in the introduction, lime and magnesia occur in Hawaiian soils in widely variable amounts, both relatively and absolutely, but generally speaking the magnesia content exceeds that of lime. The lime-magnesia ratio therefore is abnormal. For a number of years an increasing interest has been taken in this ratio in its relations to plant growth. Widely different conclusions have been reached.

The subject received one of its first important contributions from the work of Loew and May [2] in 1901. As a result of their experiments they concluded that the ratio of lime to magnesia has an important bearing upon the growth of crops. During the following years Loew and his coworkers in Japan [3] conducted further experiments along this line both in culture solutions and soil cultures, which further confirmed the conclusion arrived at formerly. As a result, the lime-magnesia ratio in soils has come to be known as the Loew theory. In general Loew found that a number of plants were considerably affected by variations in this ratio and that different ratios are best suited to the growth of different species.

Other investigators,[4] working with both field and pot cultures, have arrived at altogether different conclusions, while Voelcker,[5] after several years of careful pot experimentation, confirmed the theory so far as the growth of wheat was concerned.

From water cultures conducted at the Porto Rico Station, Gile [6] found that the concentration is of the greatest importance in determining whether the ratio of lime to magnesia exerts an influence on growth. At a low concentration he found that a wide variation in this ratio, 10:1 to 1:10, exerted no influence, while at a much higher concentration the ratio is of considerable significance. He concluded, however, that the higher concentration is rarely found in

[1] The presence of ferrous iron compounds suggests itself as being related to the inactive state of nitrification in the uncultivated soils. Hawaiian soils contain unusually large amounts of iron, a considerable portion of which exists as ferrous oxid, but the water soluble ferrous iron occurs in extremely small amounts. The difference between the cultivated and uncultivated soils in this respect is very slight. See Hawaii Sta. Bul. 30.

[2] U. S. Dept. Agr., Bur. Plant Indus. Bul. 1.

[3] Aso, Bul. Col. Agr., Tokyo Imp. Univ., 4 (1902), pp. 361–370; 5 (1903), pp. 495–499; 6 (1904), pp. 97–102; Loew and Aso, ibid., 7 (1907), pp. 395–409.

[4] Lemmermann et al., Landw. Jahrb., 40 (1911), pp. 173–254.

[5] Jour. Roy. Agr. Soc. England, 73 (1912), pp. 325–338.

[6] Porto Rico Sta. Bul. 12 (1913).

soil solutions and therefore variations in this ratio in natural soils would rarely be consequential to crops.

Concerning the correctness of this conclusion opinions may well differ, since the concentration of the real soil solution, the film moisture, is not and can hardly be known in the present state of knowledge. The principle under consideration must of necessity be worked out from culture solutions or sand cultures, since in such a complex as an ordinary soil the number of variables are entirely too numerous to permit the establishing of the principle with definiteness.

From what is known regarding the different phases of the osmotic phenomenon in their bearings on the absorption of chemical substances by plant roots, it can hardly be doubted that any considerable variation in the concentration of such elements as calcium and magnesium in nutrient solutions is likely to be attended by physiological effects, especially in certain plants.

On the other hand, the effects produced on concentration by the application of the comparatively small amounts of lime and magnesia usually employed in field experiments can only be surmised. The mere application of a given amount of soluble calcium or magnesium by no means insures a corresponding increase in the concentration of these elements in the soil solution. Therefore that widely different results have been obtained in studies with soils of different origin, composition, and properties is not surprising.

Concerning the biological phases of this question a few experiments have been conducted.

In 1904 Löhnis [1] found that the addition of magnesium carbonate to culture solutions caused a loss of ammonia from the solutions, from which he concluded that this substance is unsuited to use in nitrification studies. In 1907 Lipman and Brown [2] found that the addition of magnesium carbonate to Omelianski solutions caused a loss of ammonia during sterilization, and that upon subsequent inoculation with a soil infusion still greater losses occurred, amounting in 25 days to more than 50 per cent of the ammonia originally present. Small losses of ammonia were also sustained where calcium carbonate was used. In addition only slight nitrification took place in the solutions which contained magnesium carbonate, reaching a maximum by the sixth day followed by denitrification, whereas active nitrification took place throughout the 25-day period of observation where calcium carbonate was used. On the other hand, Owen [3] in 1908 concluded that magnesium carbonate is better suited to the stimulation of nitrification than calcium, potassium, or ammonium carbonates.

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[1] Centbl. Bakt.[etc.], 2. Abt., 13 (1904), pp. 706–715.

[2] Jour. Amer. Chem. Soc., 29 (1907), pp. 1358–1362.

[3] Georgia Sta. Bul. 81 (1908).

In 1907 Ashby [1] found that in the presence of magnesium carbonate, Azotobacter from the Rothamsted experimental plats fixed more nitrogen in mannite solutions, both in pure and mixed cultures, than in the presence of calcium carbonate. A mixture of the two carbonates proved more effective than calcium carbonate alone. The author concluded "that magnesium carbonate not only neutralizes more effectually than calcium carbonate any trace of acidity due to foreign organisms in the early stages of culture, but also prevents butyric fermentation, but at first it inhibits the growth of Azotobacter itself." In further investigation [2] he found that magnesium carbonate caused a greater loss of ammonia from ammonium sulphate solution than calcium carbonate. This loss Ashby attributed to the interaction between ammonium sulphate and the carbonates, whereby ammonium carbonate is formed, which in turn tends to volatilize from the solutions. Magnesium carbonate being more soluble than calcium carbonate, would, therefore, give rise to greater amounts of ammonium carbonate, for which reason he accounts for the greater losses in the former instances.

C. B. Lipman [3] found that, in the presence of more than very low concentrations of magnesium chlorid, the ammonification of peptone by *Bacillus subtilis* was greatly hindered, and that the simultaneous addition of varying amounts of calcium chlorid did not overcome the toxic effects. He concluded, therefore, that magnesium chlorid is toxic to the action of *B. subtilis*, and that there is no antagonism between calcium and magnesium chlorids so far as the ammonification of peptone is concerned. It should be borne in mind, however, that in general it has been found that calcium is not necessary to the growth of bacteria, and therefore, from the conception underlying Loew's theory, there need not be any antagonism between calcium and magnesium. The point of greatest interest in Lipman's experiments in this connection, however, is the fact that the magnesium salt actually proved toxic at low concentration.

In 1908 Fraps [4] found from some investigations with soils in Texas that the addition of calcium carbonate caused a greater stimulation to the nitrification of cottonseed meal than magnesium carbonate, and that a mixture of the two produced intermediate effects.

J. G. Lipman, P. E. Brown, and I. L. Owen [5] observed in 1910 that the addition of 1 gram of calcium carbonate per 100 grams of a soil from New Jersey caused a stimulation in the ammonification of dried blood, but hindered the ammonification of cottonseed meal. On the other hand, magnesium carbonate was toxic to the ammoni-

[1] Jour. Agr. Sci., 2 (1907), pp. 35–51.
[2] Idem, pp. 52–67.
[3] Bot. Gaz., 48 (1909), pp. 105–125; 49 (1910), pp. 41–50.
[4] Texas Sta. Bul. 106 (1908).
[5] New Jersey Stas. Rpt. 1910, p. 114.

fication of dried blood, but stimulated the ammonification of cotton-seed meal. In the same year, Kellerman and Robinson [1] found that the addition of magnesium carbonate to a magnesian soil in quantities greater than 0.25 per cent depressed the formation of nitrates, while calcium carbonate exerted a stimulating effect.

In investigations carried out in India in 1910 and 1911, C. M. Hutchinson [2] found that the addition of magnesium carbonate to full strength Omelianski solutions caused considerable loss of ammonia but only slight losses from dilute solutions. Furthermore, neither the neutralization of the magnesium carbonate with sulphuric acid nor the synchronous addition of calcium carbonate overcame the loss. In nitrification experiments Hutchinson found that the addition of magnesium carbonate to dilute solutions partially prevented nitrate formation for a few weeks, but at the end of 12 weeks the toxic effects had disappeared. With full-strength solutions, nitrification was greatly reduced by magnesium carbonate, and again the toxic effects were not overcome by neutralizing the magnesium carbonate. Calcium carbonate, on the other hand, did not interfere with nitrification.

In 1912 the writer [3] conducted a series of experiments on this subject, using two sandy soils from California. In the ammonification of dried blood 85 milligrams of ammonia nitrogen were formed with calcium carbonate and only 53.9 milligrams with magnesium carbonate, and no antagonism was found between the two carbonates. In nitrification studies using dried blood, calcium carbonate produced about 50 per cent stimulation, but magnesium carbonate totally inhibited nitrification. In addition to preventing nitrification, magnesium carbonate also caused slight denitrification, the original nitrate content having been reduced from 5 milligrams per 100 to 2 milligrams, where 2 grams of magnesium carbonate was added, and finally no antagonism was found between calcium and magnesium carbonates.

In view of the results previously found and the fact that, in the main, conditions differing greatly from those encountered in field studies have been employed, it becomes important to study the question further. It is of special importance to study the effects of different ratios of lime and magnesia on the various phases of bacterial action in soils, since it is now recognized that so much depends upon the biological phenomena of soils. The following investigation is offered as a contribution to the ammonification and nitrification phases of this question.

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[1] Science, n. ser., 32 (1910), p. 159.

[2] Mem. Dept. Agr. India, Bact. Ser., 1 (1912), No. 1.

[3] Univ. Cal. Pubs. Agr. Sci., 1 (1912), pp. 39–49.

EFFECTS OF CALCIUM AND MAGNESIUM CARBONATES ON AMMONIFICATION.

In experiments on this subject 100-gram portions of air-dried soils, selected so as to represent the principal types found in Hawaii, were thoroughly mixed with 2 grams of the nitrogenous materials and carbonates, then placed in tumblers, brought to optimum moisture, and covered with watch glasses. Dried blood was used as a source of nitrogen. After incubation for seven days, at from 27° to 29° C., the ammonia was determined by distillation in the usual way.

The soils used varied greatly in physical and chemical composition. No. 288 is a heavy ferruginous clay soil, containing 1.10 per cent lime and 7.94 per cent magnesia. No. 330 is a heavy clay soil from the pineapple section of the Wahiawa district. This sample contained less than 0.2 per cent of both lime and magnesia. No. 335 is coral sand soil already described. The results are shown in the following table:

Effects of calcium and magnesium carbonates on the ammonification of dried blood.

[Milligrams of ammonia nitrogen per 100 grams soil.]

Soil portions.	Carbonate added.	Soil No. 288.		Soil No. 330.		Soil No. 335.	
		Duplicates.	Averages.	Duplicates.	Averages.	Duplicates.	Averages.
1		59.9		83.4		99.3	
2	None	77.0	68.4	63.8	73.6	93.8	96.5
3	0.1 gm. $CaCO_3$	93.1		62.7			
4	0.1 gm. $CaCO_3$	77.0	85.0	65.5	64.1		
5	0.5 gm. $CaCO_3$	85.8		[1]			
6	0.5 gm. $CaCO_3$	95.5	90.6	89.0	89.0		
7	1.0 gm. $CaCO_3$	91.4		69.7			
8	1.0 gm. $CaCO_3$	87.6	89.5	83.4	71.5		
9	2.0 gm. $CaCO_3$	[1]		57.1			
10	2.0 gm. $CaCO_3$	95.0	95.0	88.2	72.6		
11	4.0 gm. $CaCO_3$	57.1		84.0			
12	4.0 gm. $CaCO_3$	59.9	58.5	108.8	96.4		
13	8.0 gm. $CaCO_3$	87.6		105.0			
14	8.0 gm. $CaCO_3$	94.9	91.2	123.2	114.1		
15	0.1 gm. $MgCO_3$	78.7		51.8			
16	0.1 gm. $MgCO_3$	94.9	86.6	79.5	65.6		
17	0.5 gm. $MgCO_3$	100.0		49.8			
18	0.5 gm. $MgCO_3$	82.0	91.0	65.8	57.8		
19	1.0 gm. $MgCO_3$	99.7		112.0		57.3	
20	1.0 gm. $MgCO_3$	[1]	99.7	121.8	116.9	64.7	62.0
21	2.0 gm. $MgCO_3$	90.4		125.3		54.0	
22	2.0 gm. $MgCO_3$	98.0	94.2	125.6	125.4	54.2	54.1
23	4.0 gm. $MgCO_3$	84.0		108.5		54.0	
24	4.0 gm. $MgCO_3$	68.6	76.3	113.4	111.4	56.8	55.4
25	8.0 gm. $MgCO_3$	64.4		89.9		49.6	
26	8.0 gm. $MgCO_3$	64.1	64.2	99.4	94.6	52.4	51.0

[1] Lost.

The above results show a wide difference in the effects produced in the different soils. In soil No. 288 the addition of calcium carbonate up to 2 per cent caused a gradational increase in ammonia formation, but with larger amounts slightly less ammonia was found. With soil No. 330, calcium carbonate in amounts less than 4 per cent produced only slight effects, while the larger amounts stimulated ammonification. These effects may be due to physical causes, since these soils are extremely heavy and the addition of the larger amounts

of calcium carbonate probably exerted an effect upon the texture so as to permit better aeration.

Magnesium carbonate [1] produced effects in soil No. 288 similar to those produced by calcium carbonate. But in soil No. 330, a reduction in the amounts of ammonia formed was caused by the smaller amounts of magnesium carbonates, while the larger amounts caused considerable stimulation. In soil No. 335, magnesium carbonate markedly decreased the amounts of ammonia that accumulated.

EFFECTS OF CALCIUM AND MAGNESIUM CARBONATES ON THE AMMONIFICATION OF DRIED BLOOD AND SOY BEAN CAKE MEAL.

Two different nitrogenous substances were employed, dried blood and soy bean cake meal, the latter of which represents the residue left after expressing the oil from the soy bean, and is produced in certain parts of the Orient in enormous quantities, where it is used both as a feed and a fertilizer. The dried blood used contained 13.29 per cent nitrogen, the soy bean cake meal 8.28 per cent. Of the soils employed, No. 9 is highly manganiferous and silty in character; No. 292 is a gravelly loam of unusually high magnesium content; No. 428 contains a large amount of organic matter and considerable amounts of calcium carbonate; No. 448 is a yellow clay soil, taken from the grounds of the Hilo Boarding School; No. 461 is clay loam taken from the rice lands of the Hanalei Valley. The other soils studied have already been discussed. After mixing with the organic nitrogenous materials and carbonates, bringing to optimum moisture, and incubation for seven days, the ammonia was determined as usual. The results are shown in the following table:

Effects of calcium and magnesium carbonates on the ammonification of dried blood and soy bean cake meal.

[Average amount in milligrams of ammonia nitrogen formed per 100 grams of soil.]

Soil portions.	Carbonate added.	Soil No. 9.		Soil No. 292.		Soil No. 428.		Soil No. 448.		Soil No. 461.		Soil No. 485.		Soil No. 487.	
		Dried blood.	Soy-bean cake.	Dried blood.	Soy-bean cake.	Dried blood.	Soy-bean cake.	Dried blood.	Soy-bean cake.	Dried blood.	Soy-bean cake.	Dried blood.	Soy-bean cake.	Dried blood.	Soy-bean cake.
1,2...	None.....	51.9	99.1	156.9	94.1	53.4	74.3	39.3	78.1	94.4	98.3	45.9	79.9	31.0	77.0
3,4...	1.0 gm. $CaCO_3$..	54.4	103.7	160.3	97.2	52.6	79.5	42.0	80.0	118.1	93.1	.....	.....	27.2	83.2
5,6...	2.0 gm. $CaCO_3$...	55.4	104.8	158.9	99.5	64.4	82.4	42.7	82.5	126.2	93.2	46.7	90.0	30.8	87.0
7,8...	4.0 gm. $CaCO_3$...	56.1	103.4	160.1	96.8	61.3	88.9	44.3	88.9	116.6	95.4	.....	.....	31.3	84.2
9,10..	1.0 gm. $MgCO_3$..	62.0	104.1	175.0	93.9	68.6	78.5	53.6	95.1	119.8	94.5	.....	.....	42.1	83.6
11,12.	2.0 gm. $MgCO_3$..	70.9	103.6	163.8	95.0	93.6	82.7	67.6	98.8	89.6	94.1	60.7	91.7	50.0	85.7
13,14.	4.0 gm. $MgCO_3$..	65.9	98.7	172.0	92.3	109.9	92.5	73.5	100.8	82.8	89.9	.....	.....	38.3	80.7
15,16.	2.0 gm. $CaCO_3$+ 2 gm. $MgCO_3$.	69.8	101.3	.....	90.7	.....	86.8	68.9	97.3	102.6	91.3	61.6	91.6	46.5	90.9
17,18.	4.0 gm. $CaCO_3$+ 2 gm. $MgCO_3$.	70.9	104.8	.....	92.4	98.7	83.4	67.6	95.9	99.7	92.4	62.2	97.7	52.4	91.1

[1] Baker's analyzed magnesium carbonate, having the composition, $3MgCO_3.Mg(OH)_2 3H_2O$, was used in these experiments. In all other instances reported in this bulletin Merck's reagent magnesium carbonate, $MgCO_3$, was used.

Considering first the calcium carbonate, it will be seen that only slight effects were produced on the ammonification of either dried blood or soy bean cake meal in soils Nos. 9, 292, 428, 448, and 487, and on the ammonification of soy-bean cake in No. 461 and that of dried blood in No. 485, while considerable stimulation resulted in the ammonification of dried blood in No. 461 and of soy-bean cake in No. 485. With the addition of magnesium carbonate, the ammonification of dried blood was stimulated in every soil except No. 461, as also was the ammonification of soy bean cake meal in Nos. 428, 448, and 485. On the other hand, magnesium carbonate produced no effects on the ammonification of soy-bean cake in soils Nos. 9, 292, and 461. In one case only—dried blood in soil No. 461—the addition of magnesium carbonate caused a decrease in the amounts of ammonia found.

In those instances where magnesium carbonates produced stimulation the further addition of calcium carbonate was without effect on this stimulation. In the one instance where magnesium carbonate proved toxic the addition of calcium carbonate, however, seems to have overcome the toxicity. It is doubtful, however, whether this is a true case of antagonism, so far as the biological processes are concerned.

The effects produced by magnesium carbonate in Hawaiian soils, therefore, proved to be quite opposite to those found in ammonification studies in solutions and in the few soils previously reported. The majority of soils used above contained an excess of magnesia over lime—No. 292 especially so—yet we find that the addition of calcium carbonate produced only slight stimulation, whereas the addition of magnesium carbonate usually caused considerable stimulation. It seems justifiable to conclude, therefore, that the lime-magnesia ratio as such has but little or no significance to the ammonification process. The above results, moreover, are in harmony with the observations of Lipman et al. in that the effects produced by magnesium carbonate depend in some instances on the nitrogenous material being acted upon; and, finally, it is of interest that magnesium carbonate caused a more marked stimulation of the ammonification of dried blood than of soy bean cake meal.

The inference has already been made that the smaller amounts of ammonia found where magnesium carbonate was added are due to the formation of ammonium carbonate and its volatilization rather than to an actual inhibition of the ammonification process. In order to throw some light on this point, further experiments were carried out with soil No. 335. In these experiments 100-gram portions were mixed with the carbonates and dried blood, then brought to optimum moisture by the addition of sterile water, and placed in wide-mouthed bottles fitted with two-hole rubber stoppers,

through which a slow current of air was slowly drawn by means of a filter pump. The air was first drawn through a solution of sulphuric acid in order to free it from all traces of ammonia, then after passing over the soil in the bottles was again drawn through sulphuric acid. After seven days' incubation the ammonia was determined, both in the soil and in the sulphuric acid. The results are shown in the following table:

Ammonification of dried blood, showing total ammonia formed.

Soil portions.	Carbonate added.	Soil 335.			
		Ammonia nitrogen found.	Ammonia nitrogen volatilized.	Totals.	Averages.
		Mg.	*Mg.*	*Mg.*	*Mg.*
1	2 gm. $CaCO_3$	56.7	17.4	74.1	
2	2 gm. $CaCO_3$	67.3	10.9	78.2	76.1
3	2 gm. $MgCO_3$	38.5	17.2	55.7	
4	2 gm. $MgCO_3$	40.5	22.4	62.9	59.3

The above data show that 14.1 milligrams of ammonia nitrogen was volatilized under the influence of calcium carbonate and 19.8 milligrams with magnesium carbonate. On the other hand, 62 milligrams accumulated in the soil where calcium carbonate was added and only 39.5 milligrams with magnesium carbonate. Combining the ammonia accumulated and that volatilized, we find that 76.1 milligrams of nitrogen was ammonified in the presence of calcium carbonate and only 59.3 milligrams in the presence of magnesium carbonate. It is thus shown that in this soil magnesium carbonate was actually toxic to ammonification. It will be recalled that the soil used is composed principally of grains of coral sand ($CaCO_3$), yet the addition of relatively small amounts of magnesium carbonate proved toxic. The conclusion, therefore, seems justifiable that magnesium carbonate is toxic to the ammonifying flora of this soil, although there are other factors that must be considered.

EFFECTS OF NATURAL LIMESTONES ON AMMONIFICATION.

Notwithstanding the theoretical interest attached to the effects produced by magnesium carbonate, it is of more practical value to determine the effects produced by the naturally occurring double carbonate of magnesium and calcium [$MgCa(CO_3)_2$], dolomite, which is present in greater or lesser amounts in practically all limestones which are now being applied to soils. Through the kindness of A. F. Whiting, of the University of Illinois, a few pounds of pulverized limestone ($CaCO_3$) and a very pure dolomite were obtained, each of which is reported as being used on a large scale.

In order to study the effects produced by these materials, both as regards stimulation and toxicity, three of the soils previously studied were employed, using also chemically pure carbonates, both singly and combined, in amounts corresponding to those in which the carbonates occur in dolomite. The results are shown in the following table:

Ammonification, showing effects produced by natural limestones.

[Average amount in milligrams of ammonia nitrogen per 100 grams soil.]

Soil portions.	Carbonate added.	Soil No. 335.		Soil No. 465.	Soil No. 516.
		Dried blood.	Soy-bean cake.	Dried blood.	Dried blood.
1,2	None	50.2	55.7	102.2	92.7
3,4	2 gm. $CaCO_3$	54.2	53.7	110.6	99.7
5,6	2 gm. $MgCO_3$	30.2	46.4	114.1	116.1
7,8	1.1 gm. $CaCO_3$+0.9 gm. $MgCO_3$	29.9	46.9	121.6	109.8
9,10	2 gm. limestone ($CaCO_3$)	50.5	56.7	108.9	94.5
11,12	2 gm. dolomite	49.3	55.4	104.6	92.2

Again, it is shown that calcium carbonate produced only slight stimulation in the ammonification of dried blood and was without effect on that of soy bean cake meal. Magnesium carbonate, on the other hand, was toxic to the ammonification of dried blood in soil No. 335 but stimulating in the other soils. The effects produced by addition of the two carbonates were similar to those produced by magnesium carbonate alone. Where the natural limestones were added, on the other hand, it will be seen that both the calcareous and the dolomitic limestones produced effects very similar to those produced by calcium carbonate. Dolomite neither proved toxic in soil No. 335 nor stimulating in soils Nos. 465 and 516. Thus it is shown that the effects produced by dolomite in no way simulated those produced by magnesium carbonate. Further discussion on this point will be made after the results from the nitrification studies have been presented.

EFFECTS OF CALCIUM AND MAGNESIUM CARBONATES ON NITRIFICATION.

In the nitrification experiments, 100-gram portions of air-dried soils, after thoroughly mixing with the nitrogenous materials and carbonates, were kept at optimum moisture in tumblers for 21 days, after which the nitrates were determined by the phenol-disulphonic acid method. Dried blood and soy bean cake meal were added at the rate of 2 grams per 100 grams of soil, and ammonium sulphate at the rate of 1.2 grams, which furnished nitrogen in an amount intermediate between those supplied by the dried blood and soy bean cake meal.

Effects of calcium and magnesium carbonates on nitrification.

[Milligrams of nitrate nitrogen per 100 grams soil.]

DRIED BLOOD, 2 GRAMS.

Soil portions.	Carbonate added.	Soil No. 292.		Soil No. 288.		Soil No. 329.		Soil No. 428.		Soil No. 448.		Soil No. 485.	
		Duplicates.	Averages.	Duplicates.	Averages.	Duplicates.	Averages.	Duplicates.	Averages.	Duplicates.	Averages.	Duplicates.	Averages.
1	None	9.3		12.3		20.0		3.2		6.5		5.0	
2	do	10.0	9.6	12.5	12.4	20.0	20.0	3.2	3.2	6.0	6.2	4.7	4.8
3	2 gm. $CaCO_3$	11.3		12.5		19.5		3.7		11.0		2.2	
4	do	10.8	11.0	12.5	12.5	20.0	19.7	3.5	3.6	9.8	10.4	2.9	2.5
5	2 gm. $MgCO_3$	5.9		6.1		18.0		2.4		7.0		1.0	
6	do	5.9	5.9	8.0	7.0	18.0	18.0	2.7	2.5	6.8	6.9	1.4	1.2
7	2 gm. $CaCO_3$ + 2 gm. $MgCO_3$.	5.9		7.0		17.5		3.0		5.5		1.4	
8	do	5.8	5.8	7.4	7.2	18.0	17.7	2.5	2.7	7.5	6.5	1.2	1.3

SOY BEAN CAKE MEAL, 2 GRAMS.

9	None	18.5		15.0		21.5		4.5		16.0		45.0	
10	do	15.0	16.7	17.5	16.2	22.0	21.7	4.6	4.5	14.0	15.0	45.0	45.0
11	2 gm. $CaCO_3$	14.5		19.0		22.0		5.0		23.5		68.0	
12	do	15.0	14.7	20.0	19.5	20.0	21.0	5.5	5.2	21.0	22.2	66.0	67.0
13	2 gm. $MgCO_3$	16.5		8.8		19.0		5.4		18.0		4.0	
14	do	20.0	18.2	9.8	9.3	19.5	19.2	3.5	4.4	20.0	19.0	3.9	3.9
15	2 gm. $CaCO_3$ + 2 gm. $MgCO_3$.	14.0		16.5		19.5		3.0		13.5		4.3	
16	do	22.0	18.0	10.5	13.2	19.5	19.5	2.5	2.7	16.5	15.0	4.3	4.3

AMMONIUM SULPHATE, 1.2 GRAMS.

17	None	2.0		4.0		17.0		1.4		3.5		4.6	
18	do	2.2	2.1	4.1	4.0	16.8	16.9	1.5	1.4	3.3	3.4	5.0	4.8
19	2 gm. $CaCO_3$	3.8		9.0		16.0		1.7		3.6		8.3	
20	do	5.5	4.6	9.2	9.1	15.0	15.5	1.5	1.6	3.9	3.7	7.3	7.8
21	2 gm. $MgCO_3$	.5		3.2		17.0		1.4		3.1		1.7	
22	do	.5	.5	3.0	3.1	16.5	16.7	1.4	1.4	3.1	3.1	1.9	1.8
23	2 gm. $CaCO_3$ + 2 gm. $MgCO_3$.	.8		3.1		16.5		1.9		3.0		2.1	
24	do	.9	.8	3.3	3.2	17.0	16.7	1.8	1.8	3.0	3.0	2.0	2.0

From these data it is shown that the nitrification of dried blood was stimulated by calcium carbonate in soils Nos. 292 and 448, while no effects were produced in soils Nos. 288, 329, and 428; magnesium carbonate, on the other hand, proved toxic in Nos. 292, 288, 428, and 485, while in soils Nos. 329 and 448 it was without effect.

In the nitrification of soy bean cake meal we find that calcium carbonate produced but little effect in soils Nos. 292, 329, and 428, but was stimulating in soils Nos. 288, 448, and 485; the addition of magnesium carbonate produced stimulation in soil Nos. 292 and 448, was without effect in No. 428, while in soils Nos. 288 and 485, particularly the latter, notably toxic effects were produced.

In the nitrification of ammonium sulphate, results somewhat different were found. Calcium carbonate caused considerable stimulation in soils Nos. 292, 288, and 485, while in Nos. 329, 428, and 448 it was without effect. Magnesium carbonates, on the other

hand, proved toxic in soils Nos. 292, 288, and 485, and exerted practically no effects in each of the other soils.

In general, the simultaneous addition of calcium and magnesium carbonates produced effects similar to those produced by magnesium carbonates alone. In the soils and with the nitrogenous materials with which calcium carbonate proved most stimulating, magnesium carbonate was most markedly toxic. Soy bean cake meal was on the whole more readily nitrified than dried blood or ammonium sulphate notwithstanding the fact that only 165 milligrams of nitrogen was added in the soy bean cake meal, while 265 milligrams was added in dried blood and 240 milligrams in the ammonium sulphate. Neither is this fact to be attributed to the lack of ammonification in the case of dried blood, for by referring to previous ammonification experiments it will be seen that vigorous ammonification of dried blood took place in these soils, and furthermore, the amount of ammonia formed in each instance was greatly in excess of the nitrate. It is possible that too great concentration of ammonium sulphate was used for the best action of the nitrifiers. On the whole the nitrifying power of these soils is rather low, especially in the case of ammonium sulphate.

NITRIFICATION IN MANGANIFEROUS SOILS.

There are considerable areas of highly manganiferous soils on Oahu on which pineapples make very poor growth. In order to throw some light on nitrification in these soils, a series of experiments was carried out, using both calcium and magnesium carbonates. The results are shown in the following table:

Effects of calcium and magnesium carbonates on nitrification in manganese soils.

[Average amount in milligrams of nitrate nitrogen per 100 grams soil.]

DRIED BLOOD, 2 GRAMS.

Soil portions.	Carbonate added.	Soil No. 514.	Soil No. 515.	Soil portions.	Carbonate added.	Soil No. 514.	Soil No. 515.
1, 2.....	None.....	28.0	6.5	7, 8.....	2 gm. $CaCO_3$ + 2 gm. $MgCO_3$.....	4.2	1.1
3, 4.....	2 gm. $CaCO_3$.....	37.0	5.2				
5, 6.....	2 gm. $MgCO_3$.....	3.5	1.1				

SOY BEAN CAKE MEAL, 2 GRAMS.

9, 10....	None.....	61.0	26.4	15, 16...	2 gm. $CaCO_3$ + 2 gm. $MgCO_3$.....	13.5	4.1
11, 12...	2 gm. $CaCO_3$.....	94.0	11.4				
13, 14...	2 gm. $MgCO_3$.....	15.2	4.1				

AMMONIUM SULPHATE, 1.2 GRAMS.

17, 18...	None.....	7.8	3.5	23, 24...	2 gm. $CaCO_3$ + 2 gm. $MgCO_3$.....	2.4	1.1
19, 20...	2 gm. $CaCO_3$.....	6.1	1.7				
21, 22...	2 gm. $MgCO_3$.....	2.5	1.1				

The above data show that nitrification takes place in the manganiferous [1] soils quite as vigorously as in other island soils. The addition of calcium carbonate caused stimulation in the nitrification of dried blood and soy bean cake meal in soil No. 514, while in soil No. 515 it caused a reduction in the nitrification of each of the materials used. In each instance magnesium carbonate proved markedly toxic, and the addition of calcium carbonate did not overcome the toxic effects.

EFFECTS OF CALCAREOUS AND DOLOMITIC LIMESTONES ON NITRIFICATION.

The following series of experiments show the effects produced by pulverized limestone and dolomitic limestone in comparison with chemically pure calcium and magnesium carbonates.

Effects of calcium and magnesium carbonates and different limestones on nitrification.

[Average amount in milligrams of nitrate nitrogen per 100 grams soil.]

Soil portions.	Carbonate added.	Soil No. 485—Soy-bean cake.	Soil No. 516—Soy-bean cake.	Soil No. 292—Dried blood.	Soil portions.	Carbonate added.	Soil No. 485—Soy-bean cake.	Soil No. 516—Soy-bean cake.	Soil No. 292—Dried blood.
1, 2...	None	44.5	13.0	9.6	9, 10..	2 gm. limestone ($CaCO_3$)	60.0	10.0	9.3
3, 4...	2 gm. $CaCO_3$	67.0	9.0	11.8	11, 12.	2 gm. dolomite	70.0	13.1	10.3
5, 6...	2 gm. $MgCO_3$	3.8	5.3	8.7					
7, 8...	1.1 gm. $CaCO_3$+0.9 gm. $MgCO_3$	8.2	6.4	9.8					

Again it will be seen that calcium carbonate produced notable stimulation in the nitrification of soy bean cake meal in soil No. 485 and a retardation in No. 516. Magnesium carbonate again proved toxic in each instance, and the simultaneous addition of calcium and magnesium carbonates, in the amounts in which they occur in dolomite, produced effects similar to those of magnesium carbonate alone. When we come to the natural limestones, it will be seen that both the calcareous and dolomitic limestones produced effects very similar to those produced by calcium carbonate, and that no toxicity was produced in any case by the dolomitic limestone.

DISCUSSION.

From the experiments above recorded, it has been shown that calcium carbonate produced only slight stimulation of the ammonification of dried blood and soy bean cake meal in most of the soils studied. Magnesium carbonate, on the other hand, caused considerable stimulation in the ammonification of dried blood in a majority of the soils, while in a number of instances the effects on the ammonification of soy bean cake meal were negligible. In two soils only,

[1] See Hawaii Sta. Bul. 26 (1912), p. 55.

Nos. 335 and 461, magnesium carbonate produced toxic effects, and in the former the effects on the ammonification of dried blood were quite similar to those found from the use of the sandy soils from California.[1] But the smaller amounts of ammonia, that accumulated in the presence of magnesium carbonate, were not entirely due to the volatilization of ammonia. Hence, magnesium carbonate was toxic to some extent. No antagonism to the action of magnesium carbonate was produced by calcium carbonate, but since magnesium carbonate is more soluble than calcium carbonate we are not justified in affirming that no significance is to be attached to the lime-magnesia ratio. It seems probable, however, that the stimulating effects produced by magnesium carbonate, on the one hand, and the toxic effects on the other, were not due to variations in this ratio, but rather to changes in the concentration of magnesium and to double decompositions.

Magnesium carbonate stimulated the ammonification of dried blood in soils which already contained abnormally high amounts of magnesium, and, since ammonia is an available form of nitrogen, the application of magnesium carbonate to these soils might prove of practical value to crops. The magnesium in these soils exists largely as hydrous silicates, and though present in much greater amounts than calcium, is considerably less soluble in dilute acids and water, and consequently is probably not present in the soil solutions in amounts equal to those of calcium. It has been shown in a different connection that Hawaiian soils have a remarkably high absorptive power for a number of chemical substances. Potassium, for instance, is fixed in relatively large amounts, but at the same time corresponding amounts of calcium and magnesium are set free.

Now, in all the soils studied above, save No. 335, it is probable that a soluble salt of magnesium (in this instance magnesium carbonate and the salts of organic acids formed through the reaction between magnesium carbonate and the organic acids set free in the decomposition of the materials added), would become fixed through double decomposition, thus setting free potassium, sodium, and calcium. Therefore, the concentration of the several constituents in the soil moisture would become greatly changed as a result of adding magnesium carbonate. Hence, the effects produced by magnesium carbonate on ammonification are probably complex, and can hardly be attributed wholly to its acting on the bacteria directly. The fact that magnesium carbonate caused no loss of ammonia in most of these soils is probably due to their fixing power for ammonia, which has been shown elsewhere to be unusually high. In every instance, except one, no antagonism to the effects produced by magnesium carbonate, either stimulating or toxic, resulted from the addition of

[1] Loc. cit.

calcium carbonate. Therefore, the effects on ammonification produced by magnesium and carbonates present a striking contrast, and the effects produced by the former suggest that the amounts of soluble magnesium in soils exerts an important bearing on bacterial action.

While magnesium carbonate produced striking effects on ammonification, the question loses much of its practical significance for the reason that magnesian limestone exerted effects similar to those brought about by calcium carbonate, and in no way comparable to those produced by magnesium carbonate.

When we come to nitrification, it was found that calcium carbonate produced stimulation in a few instances, although the increases in nitrate, with one exception, were small.[1] The carbonate content of most of these soils is low, usually less than 0.1 per cent. Generally calcium carbonate has been found to stimulate nitrification in soils which contain such low amounts of carbonate. That such is not the case in Hawaiian soils is probably due to the large amounts of aluminum and ferric hydrates present, which substances take the place of calcium carbonate in maintaining the neutral conditions as shown by Ashby.[2]

In the case of magnesium carbonate, it was found that nitrification was hindered in soils Nos. 485, 514, and 515. The toxic effects were striking, but as in the case of ammonification, practically no antagonism was found between calcium and magnesium carbonates. Dolomitic limestone, however, produced effects very similar to calcium carbonate, causing stimulation in the soils in which calcium carbonate produced stimulation, and no effects in the soils that were unaffected by calcium carbonate.

It is not possible to explain fully the action of magnesium carbonate. It seems probable, however, that the nitrifying floras of different soils would be affected differently by magnesium carbonate, being toxic in some soils and without effect in others. It will be observed by reference to the table (p. 45) that the effects produced in certain soils by magnesium carbonate depended on the nitrogenous material being acted upon. This may be accounted for, in part, by the dried blood and soy bean cake meal having reacted unequally on the growth of those organisms which feed upon ammonia and nitrates. It was observed, for instance, that wherever magnesium carbonate was added a more abundant growth of molds took place. The organic acids formed in the decay of the materials must have reacted with the magnesium carbonate, leading to the formation of different organic salts, the specific effects of which are not known. It is

[1] Peck has shown that calcium carbonate produces considerable stimulation on nitrification in some of the sugar lands of the islands. See Hawaiian Sugar Planters' Sta., Agr. and Chem. Bul. 37 (1911).

[2] Loc. cit.

probable, however, that compounds of unequal solubility and of different action on the nitrifying bacteria were formed. If so, the differences observed in the nitrification of dried blood and soy-bean cake in one and the same soil were probably due, in part at least, to causes of this nature, since the nonnitrogenous constituents of these materials differ greatly. The dried blood contained a very small nitrogen-free extract, while the soy bean cake meal contained more than 30 per cent.

That dolomite produced effects unlike those of magnesium carbonate is probably due to the insoluble nature of this material, and also to the fact that dolomite reacts with acids of various sorts less energetically even than calcium carbonate. Consequently the magnesium contained in the dolomite probably remained insoluble during the time of the experiments.

In the first series of experiments on ammonification (p. 39) Baker's analyzed magnesium carbonate, having the composition $3MgCO_3.Mg(OH_2)3H_2O$, was used. As already pointed out, considerable stimulation was produced by this material in two heavy clay soils, which stimulation was slightly greater than that produced by corresponding amounts of calcium carbonate, while the effects in a sandy soil were pronouncedly toxic. It was suggested that these results were in part referable to the magnesium hydrate contained in this material. With the hope of eliminating magnesium hydrate from consideration, Merck's reagent magnesium carbonate, which is claimed to be free from the hydrate, was used in all the subsequent experiments. This material, however, probably also contained some magnesium hydrate, as a saturated solution of it proved to be of approximately the same alkalinity to methyl orange as a saturated solution of Baker's carbonate. It is not certain, however, that the stimulation given to ammonification, as compared with that of calcium carbonate, or the toxicity found in certain instances, was due to alkalinity.

In the experiments previously reported by the writer using sandy soils from California,[1] Baker's magnesium carbonate was used, and marked toxicity, both to ammonification and nitrification, was produced. It was found, for instance, that the addition of 0.1 per cent magnesium carbonate proved toxic to a considerable degree and that 0.4 per cent produced practically maximum toxicity. Subsequently it has been found that the alkalinity of water extracts obtained by leaching portions of one of these soils after ammonification had ensued for seven days, bore no relation to the toxic or stimulating effects produced by magnesium or calcium carbonates, respectively. On the other hand, C. B. Lipman[2] has shown from

[1] Loc. cit.

[2] Centbl. Bakt. [etc.], 2. Abt., 32 (1911), pp. 58-64.

experiments with a similar sandy soil from California that considerable stimulation to ammonification was produced by the addition of 0.4 per cent sodium carbonate, a compound generally considered to be strongly alkaline. It seems probable, therefore, that the alkalinity of the magnesium carbonate was not responsible for the toxic effects in the California soils.

The difficulties inherent in the determination of actual acidity in soils are very great. Hawaiian soils, as stated above, generally have a high absorptive power for soluble bases, and the adsorptive effects and other physical phenomena that are produced when a soluble salt is added to a soil must be considered. In view of all these facts it is difficult to determine whether the greater alkalinity of the magnesium carbonate was a factor to be considered in the above experiments. It is true, however, that Hawaiian soils are potentially basic and hence it seems improbable that magnesium carbonate would cause greater stimulation to bacterial action than calcium carbonate on account of its being more actively alkaline, especially when the latter was added in amounts equal to 2 per cent of the soil. It is possible that excessive alkalinity had something to do with the marked toxicity to nitrification noted in certain instances.

SUMMARY.

(1) The pasture and forest lands of Hawaii, the soils used for aquatic crops, and most other island soils not subjected to frequent tillage contain very small amounts of nitrate but considerably larger amounts of ammonia.

(2) The uncultivated soils are capable of supporting vigorous ammonification of dried blood, but are toxic to nitrification.

(3) Nitrification takes place in Hawaiian soils after aerated conditions have been maintained for a period of several months, but not immediately following tillage. Ammonification is also stimulated by tillage.

(4) The inactive state of nitrification in the uncultivated soils is not due to the absence of the nitrifying organisms or acidity.

(5) Sterilization in the autoclave and burning failed to bring about conditions favorable to nitrification, but burning caused a splitting off of large amounts of ammonia.

(6) The beneficial effects to crops produced by burning refuse is probably due in considerable part to the formation of ammonia.

(7) The plants growing on the uncultivated soils probably absorb nitrogen largely in the form of ammonium compounds.

(8) Partial sterilization of Hawaiian soils stimulates ammonification for a short time, usually about two weeks, followed then by a retardation in ammonification. Nitrification is inhibited tem-

porarily by partial sterilization, but later on regains its activity, due possibly to reinoculation with air-borne organisms.

(9) Reinoculation of the partially sterilized with untreated soil did not overcome the stimulation to ammonification, but stimulated nitrification.

(10) A permanent increase in the available nitrogen (nitrate and ammonia) was effected by partial sterilization in certain soils, while in others the effects were very temporary. In the latter instances it is possible that nitrate and ammonia consuming organisms gained the ascendancy toward the close of the experimental periods, and that ammonification was partially inhibited by the too great accumulation of the products of bacterial action.

(11) Two-tenths per cent of toluol and carbon bisulphid were equally as effective as 4 per cent.

(12) It is believed that both the aeration and partial sterilization of Hawaiian soils bring about stimulation in bacterial action through effects produced on the colloidal soil films, but continued aeration is the more effective. The protozoan theory appears to be of doubtful application to these soils.

(13) Calcium carbonate produced considerable stimulation in the ammonification of dried blood and soy bean cake meal in certain soils; in others, only slight effects. Magnesium carbonate, on the other hand, produced marked stimulation in a number of instances. In two soils only, magnesium carbonate was toxic to ammonification. Dolomitic and calcareous limestones produced effects similar to those produced by calcium carbonate.

(14) In certain soils calcium carbonate stimulated nitrification, while in others no effects were produced. Magnesium carbonate, on the other hand, was toxic to nitrification in a majority of the soils studied.

(15) Nitrification was found to be equally as active in the manganiferous and titaniferous soils as in the other soils studied, but magnesium carbonate was especially toxic in these soils, and was more toxic to the nitrification of soy bean cake meal than of dried blood.

(16) Dolomitic and calcareous limestones produced similar effects on nitrification, bringing about stimulation in the soils in which calcium carbonate produced stimulation and no effects in the soils that were unaffected by calcium carbonate.

(17) The application of calcareous and dolomitic limestones will probably produce similar effects on the availability of nitrogen in Hawaiian soils, but regarding the effects of the burnt limes, further experiments are necessary before conclusions can be drawn.

(18) Positive conclusion can not be drawn concerning the effects of the lime-magnesia ratio on ammonification and nitrification in soils.

The evidence to date, however, points to the probability that this ratio exerts very little, if any, influence on bacterial action in the usual soil. The concentration of magnesium salts in the soil moisture, on the other hand, probably has an important influence on bacterial action.

(19) The experiments recorded in this bulletin emphasize the importance of maintaining the best aeration possible. This can not be done profitably without the rotation of crops, including green manuring. The exceedingly high clay content of much of the cultivated lands causes the soil to be very heavy, and to pack after rains, so that aeration becomes poor. By increasing the humus content aeration will be increased, drainage facilitated, and bacterial action stimulated. Thus, the plant food will become more available, deeper rooting of crops be encouraged, and their ability to withstand the effects of drought be greatly increased. No system of soil management in Hawaii can be judicious or permanent without the rotation of crops and the maintenance of humus.

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